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*RESEARCH ARTICLE: SYSTEMATIC REVIEW*

# Examining the predictive accuracy of metabolomics for small for gestational age babies: a systematic review

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## ABSTRACT

**Introduction:** To date, there is no robust enough test to predict small for gestational age (SGA) infants, which are at increased life-long risk of morbidity and mortality.

**Objective**: To determine the accuracy of metabolomics in predicting SGA babies and elucidate which metabolites were found to be predictive of this condition.

**Data sources:** Two independent researchers explored 11 electronic databases and grey literature in February 2018 and November 2018, covering publications from 1998 to 2018. Both researchers performed data extraction and quality assessment independently. Discrepancies were resolved by a third researcher.

**Study eligibility criteria**: Cohort or nested case-control studies were included, which investigated pregnant women and performed metabolomics analysis to evaluate SGA infants. The primary outcome was birthweight <10$^{th}$ centile - as a surrogate for fetal growth restriction - by population-based or customized charts.

**Study appraisal and synthesis methods**: Data on study design, obstetric variables and sampling, metabolomics technique, chemical class of metabolites, and prediction accuracy measures were extracted by two independent researchers. Authors were contacted to provide additional data when necessary.

**Results**: A total of 9,181 references were retrieved. Of these, 273 were duplicate, 8,760 were removed by title or abstract, and 133 were excluded by full text content. Thus, 15 studies were included. Only two studies used the 5$^{th}$ centile as a cutoff, and most reports sampled 2$^{nd}$ trimester pregnant women. Liquid-chromatography coupled to mass spectrometry was the most common metabolomics approach. Untargeted studies in the 2$^{nd}$ trimester provided the largest number of predictive metabolites, using maternal blood or hair. Fatty acids, phosphosphingolipids, and amino acids were the most prevalent predictive chemical subclasses.

**Conclusions and Implications**: Significant heterogeneity of participant characteristics and methods employed among studies precluded a meta-analysis. Compounds related to lipid metabolism should be validated up to the 2nd trimester in different settings.

**Systematic review registration number:** CRD42018089985.

**Keywords**: small for gestational age, fetal growth restriction, metabolomics, prediction, gas-chromatography, mass spectrometry, vitamin D, homocysteine, lipids, fatty acids.

## STRENGHTS AND LIMITATIONS OF THIS STUDY

- To our knowledge, this is the first systematic review to assess the predictive accuracy of metabolomics for an adverse pregnancy outcome.
- Using SGA as surrogate for fetal growth restriction – just as in epidemiological investigations – improves the translational potential of metabolomics.
- Identification of techniques, types of maternal samples and chemical classes paves the way for future metabolomics investigations on fetal growth patterns.
- Available data could not support a meta-analysis; further studies should include accuracy measures of individual metabolites or chemical subclasses in predicting SGA.

**ORIGINAL PROTOCOL:** Leite DFB, Morillon A-C, Melo Júnior EF, *et al*. Metabolomics for predicting fetal growth restriction: protocol for a systematic review and meta-analysis. *BMJ Open* 2018;8:e022743. doi:10.1136/bmjopen-2018-022743.

## INTRODUCTION

Fetal growth restriction (FGR) and small for gestational age (SGA) infants are major concerns in modern obstetrics. [1–3] SGA is commonly used as a proxy for FGR, [4] despite the subtle differences between these two pathological conditions. The prevalence of both varies according to criteria applied and on the population and setting, although it reaches as much as 25% in low and middle-income countries. [5] SGA newborns may have adverse health effects, such as stillbirth, [4] perinatal asphyxia, [6] impaired neurodevelopment, [7] and increased cardiovascular risk. [8,9] To date, there are no robust prediction tools for SGA using clinical factors, [10,11] ultrasound data, [12,13] or placental biomarkers. [14]

For hypothesis generating or validation purposes, metabolomics is a novel area of biomarker, discovery, development and clinical diagnostics in translational medicine. [15,16] Metabolomics is the study of all metabolites [15,16] in a given sample, i.e. low molecular weight compounds (50-2000 Da) that are intermediates of biochemical reactions and metabolic pathways, considered to directly reflect cellular activity and phenotype. [15,16] Recent studies have evaluated the pathophysiology [17–20] of SGA with metabolomics. However, little is known about the potential of metabolomics to identify predictive compounds of SGA.

Since metabolomics can identify multiple metabolites from low volume samples, and create a model from a collection of these samples, [15] it is a promising technology for hypothesis generation in a heterogeneous condition such as SGA. The prediction of SGA in pregnancy would help refer women to specialized care facilities, improving maternal and neonatal outcomes. [21,22]

In this context, the main objective of this systematic review was to assess the accuracy of metabolomics techniques in predicting SGA. As a secondary aim, we intended to determine which metabolites are predictive of this condition.

## METHODS

The protocol for this systematic review was published previously. [23] This study follows international guidelines for transparency (PROSPERO, CRD 42018089985) and respects the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement. [24] This systematic review was conducted without any public involvement, and ethical approval was unnecessary.

### Literature Search Strategy

Two independent researchers (DFBL and ACM) assessed 11 electronic databases (PubMed, EMBASE, Latin American and Caribbean Health Sciences Literature (LILACS), Scientific Electronic Library Online (Scielo), Health Technology Assessment (HTA), Database of Abstracts of Reviews of Effects (DARE), Aggressive Research Intelligence Facility (ARIF), Cumulative Index of Nursing and Allied Health Literature (CINAHL), Maternity and Infant Care (MIDIRS), Scopus, and Web of Science) and grey literature. There were no limits or language constraints; the search strategy covered published documents between 1998 and 2018. Keywords 'small for gestational age', 'metabolomics', 'prediction', 'antenatal', and variations of each, were combined with Boolean operators depending on each database requirements. The full EMBASE literature search was, as follows: ('fetal growth retardation' OR 'fetal growth restriction' OR 'intrauterine growth restriction' OR 'intrauterine growth retardation' OR 'small for gestational age') AND ('metabolomic*' OR 'metabonomic*'

OR 'metabolit* 'H NMR' OR 'proton NMR' OR 'proton nuclear magnetic resonance' OR 'liquid chromatogra*' OR 'gas chromatogra*' OR 'UPLC' OR 'ultra-performance' OR 'ultra performance liquid chromatograph*') AND ('pregnan*' OR 'antenat*' OR 'ante nat*' OR 'prenat*' OR 'pre nat*') AND ('screen*' OR 'predict*' OR 'metabolic profil*').

**Outcomes and subgroup analysis**

The primary outcome was SGA, as a surrogate for FGR and defined as birthweight <10$^{th}$ centile, by population-based or customized charts. Secondary outcomes were birthweight ≤5th or ≤3rd centile.

The intended subgroup analysis comprised: type of metabolomics technique applied (nuclear magnetic resonance, NMR; gas or liquid chromatography coupled with mass spectrometry, GC-MS or LC-MS respectively); maternal health status before pregnancy (women with *versus* without any chronic health condition); type of SGA suspected during pregnancy (early *versus* late SGA); and type of pregnancy (singleton *versus* multiple pregnancy).

**Selection Criteria of Studies, Data Collection and Analysis**

Cohort or case-control studies were included if maternal samples were collected before the clinical diagnosis of SGA, if any metabolomics technique was applied, and if the results of SGA were presented. Articles presenting data from the same research project but analyzing distinct metabolites or showing data from different countries were included. Studies were excluded (i) according to study design; (ii) if they had not applied any metabolomics technique; (iii) if they were only experimental studies; (iv) if it was not possible to extract data on SGA; (v) or if they presented

duplicate data, in which case the most complete publication was included for final analysis.

Two researchers (DFBL and ACM) independently selected studies, extracted data and discussed discrepancies. One additional reviewer (EFMJ or RTS) helped to decide, by majority, when no consensus was reached.

Piloted standardized forms were applied for data extraction, including pregnancy characteristics and experimental details. The Human Metabolome Database (HMDB) [25] and the Kyoto Encyclopedia of Genes and Genomes [26] were used for matching chemical class and metabolic pathways of each metabolite, respectively.

**Risk of bias and Assessment of concerns regarding applicability**

Two researchers (DFBL and ACM) independently evaluated individual studies using the QUADAS-2 tool. [27] One of the third reviewers (EFMJ, or RTS) helped in decision-making when no consensus was achieved.

Each study was classified as high, low, or unclear risk of bias in four Domains (Patient Selection, Index Test, Reference Standard, and Flow and Timing), and as high, low, or unclear concerns regarding applicability in the first three Domains. We did not consider two signaling questions ("Was a case-control design avoided?", "Was there an appropriate interval between the index test and reference standard?"). The nested case-control design was an inclusion criterion and maternal samples should have been collected during pregnancy, i.e. before the SGA diagnosis. Studies were considered 'low risk', for example, (i) if pregnancy or neonatal complications were not excluded in just one group of participants or data on participant selection had been provided; (ii) if methods for sample preparation and

interpretation were standardized or metabolite threshold was defined before the experiments (for targeted analysis); (iii) if the adequacy and reasons for choosing the reference birthweight chart had been explained; or, (iv) if large for gestational age babies had been excluded from the final comparative analysis.

### Data synthesis

A quantitative summary of data was performed when any predictive accuracy measures could be extracted. Authors were contacted to provide additional information, when necessary. However, only Delplancke et al [28] replied. The estimation of likelihood ratios and hierarchical summary receiver operator characteristic curve [29] were planned, as well as assessment of heterogeneity and publication bias. [30] However, due to lack of data, a meta-analysis could not be performed.

## RESULTS

### Literature search characteristics

The literature search for this systematic review was performed in February 2018, and re-run in November 2018. A total of 9,181 references were retrieved (Figure 1). After the removal of duplicate records (n=273), title and abstract screening, and analysis of the remaining 148 full-text articles, 15 articles were included. [17,18,38–42,28,31–37] See Supplementary Material 1 for excluded studies.

### Characteristics of the included studies

The characteristics of the included studies are shown in Table 1. The prevalence of SGA ranged from 7.3% [33] to 21.5% in cohort studies. [28] There were no studies

using a birthweight ≤3rd centile for a definition of SGA. The time interval between initial participant enrollment and publication varied from three [17] to 54 years, [40] although these data were unclear in 38% of the reports. [18,28,32,33,37] In nested case-control studies, participants were matched by maternal age, [17,18,38,42] ethnicity, [17,18,42] parity, [38] body mass index, [17,18,42] or infant gender. [18,38]

Participant characteristics varied between studies. Regarding gestational age at assessment, samples were collected in the 2nd trimester in one half of the studies. [17,18,33,35,37,39,42] In three reports, women were assessed at least twice. [34,38,41] In one study, maternal blood was drawn either in the 1st or 2nd trimester; [40] and in another three studies, only samples from the 3rd trimester were considered. [28,36,41] In the latter case, maternal hair was divided according to length, allowing evaluation of 2nd and 3rd trimester metabolites. [28] Studies considering the 5th centile as the cutoff, sampled women in the 1st trimester. [31,32] Twin pregnancy was a clear exclusion criterion in most studies. [17,18,31,33–35,37,40–42] Pregnancy aided by assisted reproduction [18,37] or women with pre-existing conditions [17,18,35,37,42] were also excluded, although these data were incompletely reported. [28,32,36,38,39,41] When both nulliparous and multiparous women were enrolled, there was no data analysis according to parity. Half of the studies considered term deliveries exclusively, [18,28,36,38–41] and the remaining studies did not differentiate results according to gestational age at birth.

Regarding clinical risk factors for SGA, only one paper mentioned a previous history of SGA, but findings were not adjusted for this variable. [32] All studies, except one, [28] cited participant smoking status. The rate of smoking habit ranged from 2.4% [18] to 47.5%. [40] It is important to note that Gernand et al [40] analyzed samples from women recruited between 1959 and 1965, when smoking while

pregnant was encouraged, which explains the high rate of smoking participants. The duration of smoking or any differences in birthweight (absolute measures or centiles) were not clearly stated. Although more prevalent in SGA pregnancies, results did not change with this variable control. [31,32,35,37,40] Only Gong et al [41] mentioned the suspicion of SGA in pregnancy, exhibiting decreasing abdominal circumference growth velocity between 20-36 wks. However, on final analysis, these babies were grouped with infants not suspected during pregnancy.

Table 1. Main characteristics of included studies

| Authors, year | Country, year of participants enrolment | Study design | Affected/ non-affected | Gestational age at assessment | Type of pregnancy | Parity | Birthweight curve |
|---|---|---|---|---|---|---|---|
| **Outcome: SGA <5th centile** | | | | | | | |
| Costet N et al, 2011 | France, 2002-2006 (PELAGIE Cohort) | Nested case-control | 134/ 399 | 11w | Single pregnancy | Nulliparous and parous women, unclear proportions | Customized curve |
| Ertl R et al, 2012 | United Kingdom [a] | Nested case-control | 150/ 1,000 | $11^{+0}$-$13^{+6}$w | Unclear | 55.3% nulliparous in SGA group, 48.1% nulliparous in control group | Population-based charts |
| **Outcome: SGA <10th centile** | | | | | | | |
| Grandone E et al, 2006 | Italy [a] | Cohort | 31/ 393 | 17.1 ± 1.2w[b] (mean) | Single pregnancy; no maternal pre-existing conditions | Unclear | Population-based charts |
| van Eijsden M et al, 2008 | Netherlands, 2003-2004 (ABCD Study) | Cohort | 429/ 3275 | 13.5 ± 3.3w (mean) | Term deliveries, no diabetes or hypertension | 57.6% nulliparous | Population-based charts |
| Horgan RP et al, 2011 | Australia, 2008-2011 (SCOPE Cohort) | Nested case-control | 40/ 40 | 14-16w | Single pregnancy; no other pregnancy complications | Nulliparous | Customized curve |
| Gernand AD et al, 2013 | United States, 1959-1965 (Collaborative Perinatal Project) | Nested case-control | 395/ 1751 | ≤26w | Single pregnancy; term deliveries | Parous women | Population-based charts |
| Sulek K et al, 2014 | Singapore [a] (GUSTO Study) | Nested case-control | 41/ 42 | 26-28w | Single pregnancy; term deliveries; no maternal pre-existing conditions | Nulliparous and parous women, unclear proportions | Population-based charts |
| Choi R et al, 2016 | South Korea, 2012-2013 | Cohort | 39/ 217 | 1st, 2nd or 3rd trimester | Single pregnancies | Nulliparous and parous women, unclear | Population-based charts |

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|---|---|---|---|---|---|---|---|
| | | | | | | proportions | |
| Kiely ME et al, 2016 | Ireland, 2008-2011 (SCOPE Cohort) | Cohort | 190/ 1578 | 14-16w | Single pregnancy; no maternal pre-existing conditions | Nulliparous | Customized curve |
| Ong YL et al, 2016 | Singapore [a] (GUSTO Study) | Cohort | 83/ 827 | 26-28w | Single pregnancy; no maternal chronic illness | 43.5% nulliparous | Population-based charts |
| Wang Y et al, 2016 | Taiwan, 2000-2001 (Taiwan Maternal and Infant Cohort Study) | Cohort | 35/ 188 | 3rd trimester | Unclear; term deliveries | 43% nulliparous | Population-based charts |
| Delplancke TDJ et al, 2018 | New Zealand [a] | Cohort | 20/ 73 | 34-37w | Unclear; term deliveries | Unclear | Customized curve |
| Luthra G et al, 2018 | United States, 2010-2012 (TIDES Study) | Nested case-control | 53/ 106 | 1st and 2nd trimester | Single pregnancies; term deliveries | 68% nulliparous | Customized curve |
| Gong S et al, 2018 | United Kingdom, 2008-2012 (POP study) | Nested case-control | 162/259 | 36w | Single pregnancies; term deliveries | Nulliparous | Customized curve |
| Morillon A-C et al, 2018 | 2008-2011 (SCOPE Study) | Nested case-control | 40/40 | 20w | Single pregnancies | Nulliparous | Customized curve |

[a] Unclear period of participant recruitment. [b] Mean for all study participants.

## Subgroup analysis

Due to unavailable data, the only subgroup analysis performed was related to the metabolomics approach applied (Table 2). There was no mention of adherence to metabolomics reporting data guidelines. LC-MS was the leading technique used. Three studies have investigated metabolites related to environmental exposure, from contaminated water, [31] consumer products,[36] or pesticides, [42] while others have analyzed endogenous compounds. [32–35,37–40] Only Luthra et al conducted a biomarker validation study, [38] while Gong et al [41] chose to analyze the top ten statistically different metabolites according to infant sex.

Maternal blood was the most common biological sample analyzed by LC-MS in all studies, [17,32,34–37,39–41] except for one, which used GC-MS.[39] Maternal urine was analyzed by NMR, [38] GC-MS [36] or LC-MS. [42] There was only one report using amniotic fluid [33] and two using maternal hair, [18,28] all applying GC-MS. The period of laboratory analysis was rarely specified, which made it impossible to estimate total time of sample storage.

Untargeted studies reported diverse metabolic features. Authors matched the peaks with an in-house library [18,28] or HMDB-related database. [17,42] Horgan et al [17] found 785 compounds both in maternal and newborn samples; their predictive model included 19 metabolites (only five could be putatively identified, Table 2) and used 2$^{nd}$ trimester maternal blood. Sulek et al [18] and Delplancke et al [28] prepared and analyzed samples with GC-MS using similar protocols. Sulek et al [18] identified 32 statistically different chromatographic features from which they built a predictive model using five metabolites, including two fatty acids (2-methyloctadecanoate and margarate). In contrast, Delplancke et al, [28] identified 198 metabolites, including three fatty acids (margaric, pentadecanoic, and myristic

acid) showing significantly higher levels in SGA cases, when 2nd trimester maternal hair segments were studied.

Table 2. Subgroup analysis of included studies according to which metabolomics technique was applied.

| Authors/ year | Metabolomics Technique | Maternal sample/ Storage temperature | Prediction model* | Targeted compounds | Coefficient of variation/ Limits of quantitation | Predictive compounds | Sensitivity/ Specificity | AUC |
|---|---|---|---|---|---|---|---|---|
| **Nuclear magnetic resonance** | | | | | | | | |
| Luthra G et al, 2018 | $^{1}$H-NMR 1D NOESY with pre-saturation and homonuclear 2D *J*-resolved at 300 K Bruker 600 MHz Advance III HD spectrometer | Urine/ -80°C | Targeted | Tyrosine, acetate, formate, trimethylamine | NA | None | | |
| **Gas chromatography coupled to mass spectrometry** | | | | | | | | |
| Costet N et al, 2011 | GC-MS Simple head space SPME-Capillary GC | Urine/ -20°C | Targeted | Trichloroacetic acid | <5%/ 0.01mg/L | None | 0.1/ 0.93 | |
| Sulek K et al, 2014 | GC-MS Thermo Trace GC Ultra system coupled to ISQ mass selective detector Capillary GC column: Phenomenex ZB-1701 (30 m x 250 µm id x 0.15 µm with 5 m guard column) | Hair/ -20°C | Untargeted | NA | NA | ↓ Lactate<br>↓ Levulinate<br>↑2-methyloctadecanate<br>↑Tyrosine<br>↓ Margarate | | 0.998 |
| Delplancke TDJ et al, 2018 | GC-MS: Agilent 7890B gas chromatograph, capillary column ZB-1701 (30m x 250µm id x 0.15µm with 5m guard column) 5977 A mass spectrometer, electron impact ionisation | Hair/ -20°C | Untargeted | NA | NA | ↑ Margaric acid<br>↑ Pentadecanoic acid<br>↑ Myristic acid[c] | | 0.72<br>0.73<br>0.73 |

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|---|---|---|---|---|---|---|---|---|
| **Liquid chromatography coupled to mass spectrometry** | | | | | | | | |
| Grandone E et al, 2006 | LC-MS/MS triple quadrupole Applera API 3000, TurboIonSpray ionisation | Amniotic fluid/ -80°C | Targeted | Homocysteine | Unclear | ↑Homocystine (1,29µM; 1,05-1,52M) | | |
| Horgan RP et al, 2011 | UPLC- MS/MS Thermo Fisher LTQ Orbitrap, ESI | Plasma/ -80°C | Untargeted | NA | NA | Hexacosanedioic acid, diglyceride, lyso-phosphocholine, sphinganine 1-phosphate; sphingosine 1-phosphate[d] | | 0.90 |
| Ertl R et al, 2012 | HPLC- MS/MS Shimadzu Prominence HPLC system with a column Phenomenex Luna C8 3 x 50 mm; AbSciex API-5000 triple quadrupole, ESI | Serum/ -80°C | Targeted | $25(OH)D_2$; $25(OH)D_3$ | 6.3%[a], 6.6%[b] ($D_2$); 6.5%[a], 7.3%[b] ($D_3$)/ unclear | ↓25,OH) Vitamin D (12.16ng/mL; 8.09-20.54ng/mL) | 0.72/ 0.45 | |
| Gernand AD et al, 2013 | LC-MS/MS | Serum/ -20°C | Targeted | $25(OH)D_2$; $25(OH)D_3$ | 8.2%[a] ($D_2$) 5.9%[a] ($D_3$)/ <1ng/mL | None | 0.39/ 0.66 | |
| Choi R et al, 2016 | HPLC- MS/MS Waters HPLC system, Applied Biosystems API-4000 MS/MS mass spectrometer | Serum/ -20°C | Targeted | Methylmalonic acid; homocysteine | <10%[a]; <10%[b]/ Unclear | None; | | |
| Kiely ME et al, 2016 | UPLC- MS/MS Waters Acquity UPLS system, Waters Triple Quadrupole TQD mass spectrometer | Serum/ -80°C | Targeted | $25(OH)D_2$; $25(OH)D_3$; 3-epi-$25(OH)D_3$. | <6%[a]; <5%[b]/ 0.57ng/mL ($D_2$); 0.26ng/mL ($D_3$), 0,41ng/mL (epi-$D_3$) | None | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Ong YL et al, 2016 | LC-MS/MS Applied Biosystems ThermoHypersil BDS C8 reverse-phase column | Plasma/ Unclear | Targeted | 25(OH)$D_{2;}$ 25(OH)$D_3$ | ≤10,3%[a,b]/ <1,6ng/mL | None | 0.12/ 0.87 |
| Wang Y et al, 2016 | LC-MS Agilent HPLC system, Applied Biosystems Sciex API-4000 triple quadrupole mass spectrometer | Serum/ Unclear | Targeted | PFOA; long-chain PFCA | 0,83-7,94%[a]; 1,57-24,7%[b]/ 0,07-0,45ng/mL[e] | PFDeA (OR 3,4; 95%CI 1,07-9,19), PFNDA (OR 1,83; 95% CI 1,01-3,32)[f] | |
| Gong S et al, 2018 | LC-MS/MS Shimadzu UK Limited UPLC system, ACE Excel 2 C18-PFP LC-column; Thermo Fisher Scientific Exactive orbitrap mass spectrometer | Serum/ Unclear | Untargeted | NA | | ↑N1,N12-diacetylspermine[f] | |
| Morillon A-C et al, 2018 | UPLC- MS/MS Waters Acquity UPLS system, Waters Synapt G2-S mass spectrometer | Urine/-80oC | Untargeted | NA | | None | |
| Others | | | | | | | |
| van Eijsden M et al, 2008 | GC-FID Solid phase extraction SPE, Capillary GC | Plasma/ -80ºC | Semi-targeted, Lipid extraction | Elaidic, linoleic, alfa-linolenic, eicosatetraenoic, EPA, DPA, DHA DGLA, AA, Adrenic, and Osbond acids | ≤2 - 22%[b]/ Unclear | ↓ Eicosatetraenoic acid (OR 1,5; 95% CI 1,07-2,11); ↓DPA (OR 1,45 95% CI 1,01-2,1) | |

[a]Intra-assay and [b]inter-assay coefficients of variation. [c]These metabolites were found in 2nd trimester hair segments. [d]And more 14 metabolites that could not be identified certain based on chromatographic peak and mass: Phenylacetylglutamine or formyl-N-acetyl-5-methroxykynurenamine; leucyl-leucyl-norleucine or sphingosine 1-phosphate; cervonyl carnitine and/or 1-alpha,25-dihydroxy-18-oxocholecalciferol; (15Z)-tetracosenoic acid or 10,13-dimethyl-11-docosyne-10,13-diol or trans-selacholeic acid; pencosenoic acid or cyclohexyl acetate or octanoic acid or methyl-heptenoic acid or 4-hydroxy-2-octenal or DL-2-aminooctanoic acid or 3-amino-octanoic acid; hydroxybutyrate or hydroxy-methylpropanoate or methyl methoxyacetate; lysophosphocoline and phosphocoline (more than 10 hits); phosphocoline (more than 20 hits); phosphocoline or ubiquinone-8; acetylleucil-leucil-norleucinal or oleoylglycerone phosphate or LPA(0:0/18:2(9Z,12Z)) or 1-16:1-lysoPE or phosphocoline(O-11:1(10E)/2:0) or (3s)-3,4-Di-N-hexanoyloxybutyl-1-phosphocoline or N-(3-hydroxy-propyl) arachidonoyl amine or N-methyl N-(2-hydroxy-ethyl) arachidonoyl amine or similar; lysophosphocholine (16:1) or cervonyl carnitine; preganediol-3-glucuronide or 3-alpha,20-alpha-dihydroxy-5-beta-pregnane-3-glucuronide; 6-hydroxyshingosine or

(4OH,8Z,t18:1) sphingosine or 15-methyl-15-prostaglandin D2 or 15-R-prostaglandin E2 methylester. [e]Values for all studied metabolites. [f]Predictive compounds only for female babies.

AUC: area under the receiver operating characteristic curve; $^{1}$H-NMR: hydrogen nuclear magnetic resonance; NOESY: nuclear Overhauser effect spectroscopy; GC-MS: gas chromatography coupled to mass spectrometry; SPME: solid phase micro extraction; LC-MS: liquid chromatography coupled to mass spectrometry; UPLC: ultra-performance liquid chromatography; ESI: Electrospray ionisation; FID: flame ionisation detection; PFOA: perfluorooctanoic acid; PFCA: perfluorocarboxylic acid; PFDeA: perfluorodecanoic acid; PFUnDA: perfluoroundecanoic acid; EPA: eicoisapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid; DGLA: dihomo-gama-linolenic acid; AA: arachidonic acid; OR: odds ratio; CI: confidence interval; NA: not applicable.

**Analysis of identified metabolites**

The identified compounds refer to eleven HMDB chemical classes. Fatty acids [18,28,39] comprised the most prevalent chemical class, followed by amino acids [18,33] and phosphosphingolipids [17] (Table 3).

A total of 5,974 women were assessed for vitamin D status. Results were presented as total vitamin D, [32,35,37,40] although vitamin $D_2$, $D_3$ or 3-epi-25(OH)$D_3$ [35] metabolites were measured. Results were stratified according to season of maternal sampling or latitude. Either <15ng/mL (<37.5nmol/L) [40] or <20ng/mL (<50nmol/L) [32,35,37] levels characterized vitamin D deficiency, but were statistically different in SGA pregnancies only in the 1st trimester. [32] Horgan et al found a metabolite that could represent a vitamin D derivative, but it was only predictive in combination with 18 other compounds; this model had an area under the curve (AUC) of 0.90 (optimal odds ratio (OR), 44; 95%CI 9-214). [17]

The second most frequent targeted metabolite was homocysteine, [33,34] although levels were only differentiated between normal and SGA pregnancies when measured in 2nd trimester amniotic fluid, with a multiple linear regression model $r^2$=0.012 and $p$=0.029. [33] Comparatively, the only common metabolite in 2nd trimester maternal hair was margarate, with conflicting results since it was found to be either increased (AUC 0.72, 95%CI 0.58-0.86) [28] or decreased. [18] The N1,N12-diacetylspermine and the perfluorocarboxylic acids were associated to female SGA babies, not males. The former presented a 5-fold decreased risk of SGA across quintiles. The perfluorodecanoic and perfluoroundecanoic acids presented OR of 3.14 (95%CI 1.07-9.19) and 1.83 (95%CI 1.01-3.32). [36] Tyrosine, an essential amino acid for infants, was part of the predictive model of maternal hair, combining 5 metabolites with an AUC of 0.998 (95%CI 0.992-1.0) [18]. However,

tyrosine did not predict SGA when urine samples were studied. [38] Methylmalonic acid, [34] acetate, formate, or trimethylamine, [38] did not differentiate SGA when compared to uncomplicated pregnancies ($p$>0.05).

Table 3. Predictive metabolites summarized according to their chemical class, subclass, and biological process

| Predictive metabolites | Chemical class | Chemical subclass | Metabolic pathway |
|---|---|---|---|
| Margarate | Fatty acyls | Fatty acids and conjugates | Lipid transport, metabolism, peroxidation |
| Pentadecanoic acid | Fatty acyls | Fatty acids and conjugates | Lipid transport, metabolism, peroxidation; fatty acid metabolism, fatty acid biosynthesis |
| Myristic acid | Fatty acyls | Fatty acids and conjugates | Lipid transport, metabolism, peroxidation; fatty acid metabolism, fatty acid biosynthesis |
| Eicosatetraenoic acid | Fatty acyls | Fatty acids and conjugates | Lipid transport, metabolism, peroxidation; lipid metabolism pathway |
| Docosapentaenoic acid | Fatty acyls | Fatty acids and conjugates | Lipid transport and metabolism, fatty acid metabolism, alpha linolenic acid and linoleic acid metabolisms |
| Tyrosine [a] | Carboxylic acids and derivatives | Amino acids, peptides, and analogues | Catecholamine biosynthesis; phenylalanine and tyrosine metabolism; thyroid hormone synthesis; transcription and translation |
| Homocysteine | Carboxylic acids and derivatives | Amino-acids, peptides, and analogues | Glycine and serine metabolism; methionine metabolism |
| Hexacosanedioic acid | Carboxylic acids and derivatives | Dicarboxylic acid and derivatives | Fatty acid biosynthesis |
| Sphinganine 1-phosphate | Sphingolipids | Phosphosphingolipids | Sphingolipid signalling pathway, nneuroactive ligand-receptor interaction |
| Sphingosine 1-phosphate | Sphingolipids | Phosphosphingolipids | Lipid metabolism pathway, sphingolipid metabolism |
| PFDeA | Alkyl halides | Alkyl fluorides | Not reported [b] |
| PFUnDA | Alkyl halides | Alkyl fluorides | Not reported [b] |
| 25,OH,Vitamin D | Steroids and steroids derivatives | Vitamin D and derivatives | Lipid metabolism pathway |
| Diglyceride | Glycerolipids | Diradylglycerols | Adipocytokine signaling pathway |
| Lactate | Hydroxy acids and derivatives | Alpha hydroxy acids and derivatives | Gluconeogenesis, glycogenosis types IB and IC, pyruvate metabolism, triosephosphate isomerase |
| N1,N12-diacetylspermine | Carboximidic acids and derivatives | Carboximidic acids | |
| Lyso-phosphocholine | Glycerophospholipids | Glycerophosphocholines | Not reported [b] |
| 2-methyloctadecanate | Saturated hydrocarbons | Alkanes | Not reported [b] |
| Levulinate | Keto acids and derivatives | Gamma-keto acids and derivatives | Not reported [b] |

[a] Essential amino acid for infants. [b] No human metabolic pathways reported at KEGG. PFDeA: perfluorodecanoic acid; PFUnDA: perfluoroundecanoic acid.

**Risk of bias and Applicability Concerns**

Figure 2 shows synthesized data for all included studies. See Supplementary Material 2 for individual QUADAS-2 data.

Regarding the risk of bias, all cohort studies conducted a consecutive participant inclusion. [28,33–37,39] Nested case-controls matched cases and controls randomly [33–35,41] or according to maternal and infant characteristics. [17,18,38,42] One study [41] failed to mention matching procedures ('Patient Selection' domain). Researchers were not blinded to SGA status when interpreting metabolomics results, [17,18,41,28,32,35–40] and thresholds of targeted metabolites were not pre-specified [31,33,36,38,39] ('Index Test' domain). Conversely, SGA identification was not influenced by the metabolomics test, although it was unclear when laboratory experiments were performed in some studies. [18,28,31,33,34,41] Birthweight charts were adequate, except for two studies. The first did not report which centile was chosen, [18] and the second used a centile designed for a different population [33] ('Reference Test' domain). Two studies were ranked as 'high risk' because not all participants were included in the analysis [31,37] ('Flow and Timing' domain).

The QUADAS-2 tool also highlights the importance of how the findings of the included studies are suitable to the review question. In the Patient Selection domain, it was ranked as 'high applicability concerns' when infants born between the 4$^{th}$ and the 10$^{th}$ centile, but with normal abdominal circumference growth velocity, were not included in final analysis. [41] It was 'unclear' when the gestational age of maternal assessment was not standardized, [34] or was inferred by hair segment length; [28] or when few metabolites from untargeted studies were chosen for interpretation [41]

('Index Test' domain). Finally, it was 'high' when the birthweight charts applied did not correspond to the study population [18,33] ('Reference Standard' domain).

**Meta-analysis**

From the 15 included studies, only three were designed for prediction purposes [17,18,42] and provided the AUC. The remaining reports described statistical differences of metabolites between SGA pregnancies and controls. [28,31,40,41,32–39] Accuracy measures were extracted when available (Table 2). However, due to marked heterogeneity (Tables 1 and 2) of gestational age at sampling, type of samples used, type of birthweight chart chosen, thresholds for vitamin D deficiency, metabolomics approach, and identified compounds, a meta-analysis could not be performed.

## DISCUSSION

### Main findings

In this first systematic review of metabolomics and adverse pregnancy endpoints, we presented techniques and metabolites, which were studied for the prediction of SGA. Any effect on birthweight has important implications for perinatal research, since it is related to short and long-term outcomes, [43–46] and in different generations. [47,48] Intrauterine environment influences fetal growth through epigenetic processes: altered gene expression potentially leads to distinct phenotypes. [49] Metabolomics is the most adequate approach to study this outcome, since it is most directly related to phenotype. [50]

Interpretation of metabolomics findings in pregnancy can be challenging. Firstly, maternal metabolites concentrations are influenced by placental transfer to

and from the fetus. The 'mirror effect', seen for maternal plasma and venous cord blood metabolites at birth, [51] cannot be ruled out when only maternal specimens are studied. Secondly, maternal exposure to distinct compounds may affect metabolite levels. Statistically significant differences between SGA infants and controls may not express the totality of underlying pathological pathways and have no clinical meaning. Finally, it is unclear when the processes leading to SGA are initiated. The disruption in maternal metabolism can theoretically occur at any time. In general the lower the gestational age at which the condition is suspected, the more severe the phenotype will be at birth. [52,53] Thus, the description of clinical data in translational studies must deal with all these confounding factors.

Gestational age at sampling is probably the most important parameter for prediction purposes. With timely prediction, women could be referred to specialized care, have increased surveillance, and this in turn may lead to a reduction in perinatal mortality. There are temporal changes in the maternal metabolome during pregnancy; [28,54–57] therefore, it is reasonable to expect distinctive metabolites at different stages of pregnancy, as reported here. Unfortunately, a wide or unclear definition of gestational age of sampling [34,36,38,40] render a more precise interpretation impossible, and may limit the clinical application of these results.

In contrast, gestational age at birth and birthweight centile seem to be the hallmarks of severity and prognosis of growth restriction. [6,58] Indeed, term and preterm SGA babies show distinct clinical phenotypes, and there are concerns that some babies <10$^{th}$ centile of birthweight are constitutionally small infants. [59–61] If only term deliveries are evaluated, the most severe cases of growth restriction may be potentially missed. Moreover, when term and preterm births are analyzed together, or when lower cutoffs are not specified (e.g. ≤3$^{rd}$ or ≤5$^{th}$ centile), the lack of

predictive metabolites might mean that they are distinct conditions. Thus, we hypothesize that the predictive performance of metabolomics may be improved if data is analyzed by gestational age at delivery, and by different cutoffs of birthweight centiles.

Evidence suggests that tobacco smoke has an impact on birthweight, [62–64] although it is uncertain how and when fetal growth is impaired. It is possibly related to oxidative stress, [65] and both maternal and fetal metabolism may be disturbed at delivery. [66,67] Studies that were included did not investigate cigarette-related chemicals or quantify exposure to tobacco smoke. Therefore, no relationship between SGA and tobacco was found. Hence, we suggest that tobacco interferes with ongoing metabolic pathological processes, or its disturbance is related to additional metabolic pathways other than the one examined by the included studies.

**Subgroup and metabolite findings**

No reports have explored data on any maternal chronic condition, suspicion of SGA in pregnancy, or number of fetuses. The lack of clear statements about participant selection have hindered data interpretation and precluded these analyses.

The majority of included studies performed a targeted approach, i.e. a hypothesis-testing evaluation, [16,50] driven by epidemiological or experimental data regarding SGA newborns. None of the targeted metabolites [31–40] were in common with those found by 'hypothesis-generating' metabolic profiling [17,18,28,41,42] investigations. This reinforces the suggestion that various maternal metabolic pathways may be triggered by the SGA condition, and be detected by different biological samples. However, since blood is a very complex sample and GC-MS only

evaluates volatile molecules, [50] therefore our findings may be biased by study methodologies.

Untargeted studies, as expected, have characterized several metabolites that may be validated in future investigations. Nine lipids and fatty acid metabolites, [17,18,28,39] two amino acids, [18,33] and a steroid [17,32] have been identified as potential biomarkers of SGA.

All lipid-related metabolites identified are intermediates for energy storage and breakdown. Most metabolites were found in maternal blood [17] or hair of the SGA group. [18,28] Blood levels of saturated and monounsaturated non-esterified fatty acids apparently remain stable throughout pregnancy, while long chain polyunsaturated fatty acid (DHA and EPA, for example) measurements seem to show ethnicity-related changes. [57] Experimental data shows the importance of hypoxia and oxidative stress to placental function and ultimately, to birthweight. [68,69] Findings from included studies may represent a dysregulation of lipid pathways at the placental level, but an association with maternal background is unclear. Therefore, we hypothesize that disorders of lipid metabolism may be the 'metabolic snapshot' of defective deep placentation, [70] and might reflect maternal efforts to respond to impaired fetal growth.

Recommendations on the assessment of vitamin D and cutoffs to define vitamin D deficiency in pregnancy are controversial. [71] However, vitamin D supplementation decreases SGA risk. [72] In early pregnancy, vitamin D status has been related to SGA, [73,74] which is in accordance with this review, despite the inconsistent findings. [75] There is evidence that trophoblasts actively produce and secrete vitamin D metabolites, [76] but it is not clear how they mediate fetal growth impairment. Altered hepatic gene expression and liver function in vitamin D deficient

female rats, [77] and single nucleotide polymorphisms [78] in vitamin D receptor gene have been suggested as mechanisms to be explored by a multidimensional omics approach.

Finally, homocysteine is an intermediate metabolite of the folate cycle. It is indirectly involved with DNA methylation and is a marker of folate deficiency. [79] Maternal levels rarely reach hyperhomocysteinemia limits, [80] but folate depletion [81–83] and homocysteine itself[80] are thought to be associated with a higher SGA risk. In this review, homocysteine was only statistically different in SGA pregnancies when measured in amniotic fluid, [33] although within the normal ranges proposed for 17-21 weeks. [84] Since amniocentesis is generally performed in women at higher obstetrical risk, future studies should investigate whether homocysteine in amniotic fluid represents a confounding factor or a new biomarker. [85]

**Methodological quality**

Most studies were ranked as 'low risk' of bias or applicability to the review question. However, the lack of clear descriptions of laboratory experiments, including sample preparation and storage, and blinding of the researchers to the case/control status, are major pitfalls of the included studies.

**Strengths and limitations**

To our knowledge, this is the first systematic review of metabolomics and an adverse pregnancy outcome (SGA). We presented possible biomarkers of SGA pathophysiology, metabolites implicated in lipid transport and metabolic pathways, as well as gluconeogenesis.

However, this analysis has some limitations. First, included studies showed heterogeneity, which is fundamental in systematic reviews. Indeed, there was a wide variety of participant characteristics and methods used, and not all authors provided a detailed description of methods employed. Although the Metabolomics Standard Initiative was released in 2007, [86] there is still poor adherence to guidelines. [87,88] Clear reporting [15,87,88] and data sharing in repositories are crucial steps in identifying features of interest, specifically possible biomarkers to be validated in the clinical studies. [15] Secondly, we could not perform a meta-analysis of the extracted data, impacting the translational potential of metabolomics.

Thirdly, we considered that birthweight was a surrogate measure of intrauterine development. SGA and FGR are not interchangeable concepts. However, SGA has been used as a surrogate for FGR in many clinical studies due to difficulties in defining optimal intrauterine growth: (i) FGR diagnosis relies mostly on ultrasound measurements of fetal biometry, [3,89] which in turn is subject to systematic errors; [90] (ii) intrauterine development is adaptive, rather than uniform [91] or only genetically driven; [49] (iii) growth impairment at birth better identifies adverse neonatal outcomes than during pregnancy. [58] It is recognized that changes in obstetric care occur when growth restriction is suspected, and neonatal outcomes are improved. [21,22] Thus, an accurate prediction of SGA during pregnancy will be a turning point in modern obstetrics.

## CONCLUSIONS AND IMPLICATIONS FOR PRACTICE

Using the available clinical tools, efforts to predict SGA remain disappointing. Since SGA is a heterogeneous condition, it benefits from metabolomics. This novel area of research allows analysis of numerous types of biological fluids and detects

thousands of metabolites in complex samples. [15,16,25] However, findings of this systematic review must be interpreted with caution. The type of samples used may have influenced LC-MS (2nd trimester maternal blood) and GC-MS (2nd trimester maternal hair) findings in individual studies. Furthermore, the prediction of SGA in the context of maternal disorders, suspected FGR and twin pregnancies is an open field for future metabolomics studies, and environmental exposure investigation as well.

Surprisingly, none of the studies used ≤3rd centile of birthweight as a cutoff or analyzed preterm deliveries and hypertensive syndromes. Considering our findings and the different phenotypic manifestations of SGA, we envision a better performance when (i) cutoffs other than the 10th centile are tested; (ii) data on gestational age at sampling and at birth are standardized; and (iii) other pregnancy-related syndromes are considered, especially hypertension. Thus, future metabolomics results should advance in these critical points.

Finally, all detected biomarkers were related to lipid pathways and energy metabolism. We consider that research efforts to predict SGA should focus on compounds involved in these pathways, up to the 2nd trimester of pregnancy.

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## AUTHORS CONTRIBUTIONS

DFBL and ACM have equally contributed to this report, and both are guarantors of this review. They elaborated the protocol, searched the literature, selected studies, extracted data, assessed risk of bias, and drafted the initial manuscript. RTS and EFMJ have participated in judging inclusion of studies, interpreting data, and revising the manuscript. FM have supported data extraction and have critically examined the clinical interpretation of results. ASK has discussed the quantitative data synthesis, and supervised the report writing. PNB, LCK, and JGC have supervised and approved all steps. All authors have read and agree with this submission.

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## COMPETING INTERESTS

None to declare.

## PROVENANCE AND PEER REVIEW

Not commissioned; externally peer reviewed.

## Figure captions

Figure 1. PRISMA flowchart of study identification, screening and selection. From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097. For more information, visit www.prisma-statement.org.

Figure 2. Assessment of risk of bias (A) and applicability concerns (B) of individual studies.

## Supplementary material description

Supplementary material 1 – List of excluded studies and reasons.

Supplementary material 2 - Individual QUADAS-2 data for all 15 included studies.

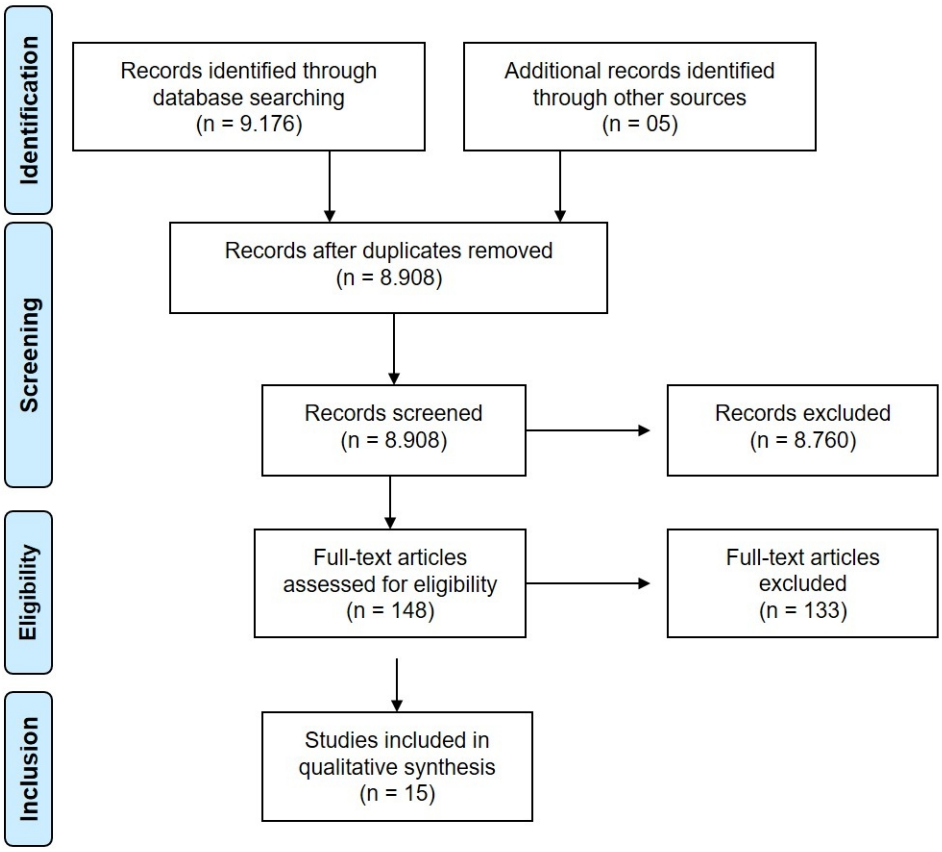


Figure 1

176x155mm (150 x 150 DPI)

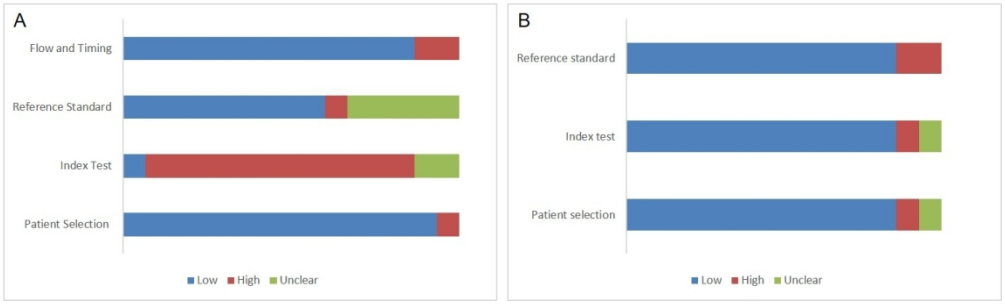


Figure 2

257x76mm (150 x 150 DPI)

**Examining the predictive accuracy of metabolomics for small for gestational age babies: a systematic review**

Debora F. B. Leite & Aude-Claire Morillon, Elias F. Melo Júnior, Renato T. Souza, Fergus P. McCarthy, Ali S. Khashan, Philip N. Baker, Louise C. Kenny, José Guilherme Cecatti.

Supplementary material 1 – List of excluded studies and reasons.

| Authors/ year | Country of enrollment | Additional comments |
|---|---|---|
| *Exclusions according to study design or statistical analysis* | | |
| Barnes CM et al, 2010 | United States | Maternal samples collected at delivery. |
| Bobinski R. 2013 | Poland | Cross-sectional study. |
| Bobinski R. 2014 | Poland | Cross-sectional study. |
| Cao WC et al, 2016 | China | Cross-sectional study. The metabolomics technique was not applied. |
| Chen TT et al, 2017 | China | Cross-sectional study. |
| Cinelli et al, 2018 | Italy | |
| D'Anna R et al, 2004 | Italy | Cross-sectional study. The metabolomics technique was not applied. |
| Guo H et al, 2014 | China | Cross-sectional study. |

| | | |
|---|---|---|
| Guo J et al, 2016 | China | Cross-sectional study. |
| Maekawa R et al, 2017 | Japan | Cross-sectional study. |
| Mao D et al, 2010 | China | Cross-sectional study. |
| Miranda J et al 2018 | Spain | Cross-sectional study. |
| Powell et al, 2018 | Australia | SGA babies not suspected before birth were considered healthy infants. |
| Spanou L. et al, 2017 | Greece | Cross-sectional study. |
| Stein TP et al, 2008 | United States | Newborns with birth defects were included in the analysis. |
| Tang R et al, 2013 | China | Cross-sectional study. |
| Visentin S et al, 2017 | Italy | Maternal samples collected after clinical recognition of FGR/SGA. |
| Zhu Y et al, 2018 | China | Cross-sectional study. |
| Zota AR et al, 2009 | United States | Cross-sectional study. The metabolomics technique was not applied. |
| | | |
| *Studies that have not applied metabolomics technique* | | |
| Baker PN, 2009 | United Kingdom | |
| Berkowitz GS et al, 2004 | United States | |
| Bodnar LM et al, 2012 | United States | |

| | | |
|---|---|---|
| Braun JM et al, 2011 | United States | There is no data about FGR. |
| Cetin I et al, 2002 | Italy | |
| Chong MFF et al, 2015 | Singapore | There is no data about birth weight. |
| Colapinto CK et al, 2015 | Canada | The metabolomics technique was not applied on pregnant women's specimens. |
| Cupul-Uicab LA et al, 2013 | United States | |
| Fruscalzo A et al, 2015 | Italy | There is no data about birth weight. |
| Jusko TA et al, 2006 | United States | |
| Koepke R et al, 2004 | Mexico | |
| Lõpez-Alarcõn M et al, 2015 | Mexico | There is no data about birth weight. |
| Maruta E et al, 2017 | Japan | |
| Miranda ML et al, 2015 | United States | |
| Morley R et al, 2006 | Australia | |
| Muthayya S et al, 2006 | India | |
| Paşaoğlu H et al, 2003 | Turkey | |
| Rahman A et al, 2009 | Bangladesh | |
| Rajasingam D et al, 2009 | United Kingdom | |

| | | |
|---|---|---|
| Savitz DA et al, 2002 | United States | The metabolomics technique was not applied for pregnant women's specimens. |
| Savvidou MD et al, 2003 | United Kingdom | |
| Schneuer FJ et al, 2014 | Australia | |
| Snijder CA et al, 2013 | Netherlands | |
| Sweeney AM & Symanski E, 2007 | United States | |
| Takimoto H et al, 2007 | Japan | |
| Terrell ML et al, 2015 | United States | |
| Wei Y et al, 2017 | Bangladesh | |
| Weisskopf MG et al, 2005 | United States | |
| Whyatt RM et al, 2009 | United States | |
| Xue F et al, 2007 | United States | |
| | | |
| *Studies that have not presented specific data about FGR/SGA* | | |
| Bach CC et al, 2016 | Denmark | |
| Bachkangi P et al. | United Kingdom | |
| Bahado-Singh RO et al, 2012 | United Kingdom | |

| | |
|---|---|
| Bahado-Singh RO et al, 2015 | United Kingdom |
| Bahado-Singh RO et al, 2017 | United Kingdom |
| Bentley-Lewis R, 2015 | United States |
| Braun JM et al, 2009 | United States |
| Buckley JP et al, 2016 | United States |
| Cantonwine D et al, 2010 | Mexico |
| Cantonwine D et al, 2015 | United States |
| Casas M et al, 2016 | Spain |
| Castorina R et al, 2017 (a) | United States |
| Chou WC et al, 2014. | Taiwan |
| Cunha Figueiredo AC et al, 2017 | Brazil |
| Dalsager L et al, 2018 | Denmark |
| De Renzy-Martin KT. et al, 2014 | Poland |
| Debost-Legrand A et al, 2016 | France |
| Desert et al, 2015 | France |

| | |
|---|---|
| Diaz SO et al, 2011 | Portugal |
| Diaz SO et al, 2013 | Portugal |
| Dobierzewska A et al, 2017 | Chile |
| Dudzik D et al, 2015 | Spain. |
| Engström KS et al, 2010 | Bangladesh |
| Ettinger AS et al, 2017 | Canada |
| Feng L et al, 2016 | China |
| Ferguson KK et al, 2014 | United States |
| Ferguson KK et al, 2015 | United States |
| Ferguson KK et al, 2017 | United States |
| Finkelstein JL et al, 2015 | United States |
| Fischer ST et al, 2017 | United States |
| Gao H et al, 2017 | China |
| Gardner RM et al, 2011 | Bangladesh |
| Ghartey J et al, 2017 | United States |
| Graça G et al, 2010 | Portugal |

| | | |
|---|---|---|
| Graça G et al, 2012 | Portugal | |
| Graça G et al, 2012 (b) | Portugal | |
| Hogeveen M et al, 2010 | Netherlands | |
| Huang J et al, 2017 | China | |
| Kalhan SC et al, 2003 | United States | |
| Khalil AA et al, 2013 | United Kingdom | |
| Kuc S et al, 2014 | Netherlands | |
| Lenters V et al, 2013 | Greenland, Poland, Ukraine | |
| Lenters V et al, 2016 | Greenland, Poland, Ukraine | |
| Liu K et al, 2017 | China | |
| Lopez-Espinosa MJ et al, 2015 | Spain | |
| Marchlewicz EH et al, 2016 | United States | |
| Minatoya M et al, 2017 | Japan | |
| Minatoya M et al, 2017 (b) | Japan | |
| Minatoya M et al, 2018 | Japan | |
| Murphy MM et al, 2007 | Spain | There is no data about any pregnancy outcomes |

| | | |
|---|---|---|
| Odibo AO et al, 2011 | United States | |
| Pinney SE et al, 2017 | United States | |
| Polanska K et al, 2014 | Poland | |
| Polanska K et al, 2014 (b) | Poland | |
| Porter A et al, 2018 | United States | |
| Rejc B et al, 2016 | Slovenia | |
| Rijvers CAH et al, 2013 | Netherlands | |
| Robledo C et al, 2013 | United States | |
| Sachse D et al, 2012 | Norway | |
| Scholtens DM et al, 2016 | United Kingdom | |
| Shisler S et al, 2017 | United States | Not all analysis were performed with metabolomics approach. |
| Tamblyn JA et al, 2018 | Ireland | Duplicate data. Check Kiely ME et al, 2016. |
| Thomas MM et al, 2015 | New Zealand | |
| Van Lee L et al, 2015 | Singapore | |
| Virgiliou C et al, 2017 | Greece | |
| Walsh J et al, 2012 | Ireland | |

| | | |
|---|---|---|
| Wang PW et al, 2015 | Taiwan | |
| Watkins DJ et al, 2016 | United States | |
| Wolff MS et al, 2008 | United States | |
| Woods MM et al, 2017 | United States | |
| Yang P et al, 2018 | China | |
| | | |
| *Duplicate data* | | |
| Horgan R et al, 2009 | Australia | Check Horgan R et al, 2011. |
| Horgan R et al, 2011 | Australia | Check Horgan R et al, 2011. |
| Khashan AS et al, 2013 | Ireland | Check Kiely ME et al, 2016. |
| Sulek et al, 2014 | Singapore | Check Sulek et al, 2014. |

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Supplementary material 2 - Individual QUADAS-2 data for all 15 included studies.

| Studies | Risk of bias | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Patient selection | | Index test | | Reference standard | | Flow and timing | |
| | Was a consecutive or random sample of patients enrolled? | Did the study avoid inappropriate exclusions? | Were the index test results interpreted without knowledge of the results of the reference standard? | If a threshold was used, was it pre-specified? | Is the reference standard likely to correctly classify the target condition? | Were the reference standard results interpreted without knowledge of the results of the index test? | Did all patients receive the same reference standard? | Were all patients included in the analysis? |
| Grandone E et al, 2006 | Yes | Yes | Unclear | No | No | Unclear | Yes | Yes |
| van Eijsden M et al, 2008 | Yes | Yes | No | No | Yes | Yes | Yes | Yes |
| Horgan R et al, 2011 | Yes | Yes | No | Yes | Yes | Yes | Yes | Yes |
| Costet N et al, 2012 | Yes | Yes | Yes | No | Yes | Unclear | Yes | No |
| Ertl R et al, 2012 | Yes | Yes | No | Yes | Yes | Yes | Yes | Yes |
| Gernand AD et al, 2013 | Yes | Yes | No | Yes | Yes | Yes | Yes | Yes |
| Sulek K et al, 2014 | Yes | Yes | No | Yes | Unclear | Unclear | Yes | Yes |
| Choi R et al, 2016 | Yes | Yes | Unclear | Yes | Yes | Unclear | Yes | Yes |
| Kiely ME et al, 2016 | Yes | Yes | No | Yes | Yes | Yes | Yes | Yes |
| Ong YL et al, 2016 | Yes | Yes | No | Yes | Yes | Yes | Yes | No |
| Wang Y et al, 2016 | Yes | Yes | No | No | Yes | Yes | Yes | Yes |
| Delplancke TDJ et al, 2018 | Yes | Yes | No | Yes | Yes | Unclear | Yes | Yes |
| Luthra G et al, 2018 | Yes | Yes | No | No | Yes | Yes | Yes | Yes |
| Gong S et al, 2018 | No | Yes | No | No | Yes | Unclear | Yes | Yes |
| Morillon AC et al, 2018 | Yes | Yes | No | Yes | Yes | Yes | Yes | Yes |

| Studies | Applicability concerns | | |
|---|---|---|---|
| | Patient selection | Index test | Reference standard |
| | Are there concerns that the included patients do not match the review question? | Are there concerns that the index test, its conduct, or interpretation differ from the review question? | Are there concerns that the target condition as defined by the reference standard does not match the review question? |
| Grandone E et al, 2006 | No | No | Yes |
| van Eijsden M et al, 2008 | No | No | No |
| Horgan R et al, 2011 | No | No | No |
| Costet N et al, 2012 | No | No | No |
| Ertl R et al, 2012 | No | No | No |
| Gernand AD et al, 2013 | No | No | No |
| Sulek K et al, 2014 | No | No | Yes |
| Choi R et al, 2016 | Unclear | No | No |
| Kiely ME et al, 2016 | No | No | No |
| Ong YL et al, 2016 | No | No | No |
| Wang Y et al, 2016 | No | No | No |
| Delplancke TDJ et al, 2018 | No | Unclear | No |
| Luthra G et al, 2018 | No | No | No |
| Gong S et al, 2018 | Yes | Yes | No |
| Morillon AC et al, 2018 | No | No | No |

# PRISMA 2009 Checklist

**Examining the predictive accuracy of metabolomics for small for gestational age babies: a systematic review**

Debora F. B. Leite & Aude-Claire Morillon, Elias F. Melo Júnior, Renato T. Souza, Fergus P. McCarthy, Ali S. Khashan, Philip N. Baker, Louise C. Kenny, José Guilherme Cecatti

| Section/topic | # | Checklist item | Reported on page # |
|---|---|---|---|
| **TITLE** | | | |
| Title | 1 | Identify the report as a systematic review, meta-analysis, or both. | 1 |
| **ABSTRACT** | | | |
| Structured summary | 2 | Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number. | 3-4 |
| **INTRODUCTION** | | | |
| Rationale | 3 | Describe the rationale for the review in the context of what is already known. | 5 |
| Objectives | 4 | Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS). | 6 |
| **METHODS** | | | |
| Protocol and registration | 5 | Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number. | 6 |
| Eligibility criteria | 6 | Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale. | 7-8 |
| Information sources | 7 | Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched. | 6-7/ 9 |
| Search | 8 | Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated. | 6-7 |
| Study selection | 9 | State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis). | 7-8 |
| Data collection process | 10 | Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators. | 8 |
| Data items | 11 | List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made. | 7-8 |
| Risk of bias in individual studies | 12 | Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis. | 8-9 |

# PRISMA 2009 Checklist

**Examining the predictive accuracy of metabolomics for small for gestational age babies: a systematic review**

Debora F. B. Leite & Aude-Claire Morillon, Elias F. Melo Júnior, Renato T. Souza, Fergus P. McCarthy, Ali S. Khashan, Philip N. Baker, Louise C. Kenny, José Guilherme Cecatti

| | | | |
|---|---|---|---|
| Summary measures | 13 | State the principal summary measures (e.g., risk ratio, difference in means). | 9 |
| Synthesis of results | 14 | Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., $I^2$) for each meta-analysis. | 9 |



| Section/topic | # | Checklist item | Reported on page # |
|---|---|---|---|
| Risk of bias across studies | 15 | Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies). | 8-9 |
| Additional analyses | 16 | Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified. | 7 |
| **RESULTS** | | | |
| Study selection | 17 | Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram. | 9 |
| Study characteristics | 18 | For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations. | 9-13 |
| Risk of bias within studies | 19 | Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12). | 23-24 |
| Results of individual studies | 20 | For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot. | 20-22 |
| Synthesis of results | 21 | Present results of each meta-analysis done, including confidence intervals and measures of consistency. | 24 |
| Risk of bias across studies | 22 | Present results of any assessment of risk of bias across studies (see Item 15). | 9; 23-24 |
| Additional analysis | 23 | Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]). | NA |
| **DISCUSSION** | | | |
| Summary of evidence | 24 | Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers). | 24-28 |
| Limitations | 25 | Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias). | 28-29 |
| Conclusions | 26 | Provide a general interpretation of the results in the context of other evidence, and implications for future research. | 29-30 |
| **FUNDING** | | | |

# PRISMA 2009 Checklist

**Examining the predictive accuracy of metabolomics for small for gestational age babies: a systematic review**

Debora F. B. Leite & Aude-Claire Morillon, Elias F. Melo Júnior, Renato T. Souza, Fergus P. McCarthy, Ali S. Khashan, Philip N. Baker, Louise C. Kenny, José Guilherme Cecatti

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|---|---|---|---|
| Funding | 27 | Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review. | 38-39 |

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BMJ Open

# Examining the predictive accuracy of metabolomics for small for gestational age babies: a systematic review

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| | |

*RESEARCH ARTICLE: SYSTEMATIC REVIEW*

# Examining the predictive accuracy of metabolomics for small for gestational age babies: a systematic review

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## ABSTRACT

**Introduction:** To date, there is no robust enough test to predict small for gestational age (SGA) infants, which are at increased life-long risk of morbidity and mortality.

**Objective**: To determine the accuracy of metabolomics in predicting SGA babies and elucidate which metabolites are predictive of this condition.

**Data sources:** Two independent researchers explored 11 electronic databases and grey literature in February 2018 and November 2018, covering publications from 1998 to 2018. Both researchers performed data extraction and quality assessment independently. A third researcher resolved discrepancies.

**Study eligibility criteria**: Cohort or nested case-control studies were included, which investigated pregnant women and performed metabolomics analysis to evaluate SGA infants. The primary outcome was birthweight <10$^{th}$ centile - as a surrogate for fetal growth restriction - by population-based or customized charts.

**Study appraisal and synthesis methods**: Two independent researchers extracted data on study design, obstetric variables and sampling, metabolomics technique, chemical class of metabolites, and prediction accuracy measures. Authors were contacted to provide additional data when necessary.

**Results**: A total of 9,181 references were retrieved. Of these, 273 were duplicate, 8,760 were removed by title or abstract, and 133 were excluded by full text content. Thus, 15 studies were included. Only two studies used the 5$^{th}$ centile as a cutoff, and most reports sampled 2$^{nd}$ trimester pregnant women. Liquid-chromatography coupled to mass spectrometry was the most common metabolomics approach. Untargeted studies in the 2$^{nd}$ trimester provided the largest number of predictive metabolites, using maternal blood or hair. Fatty acids, phosphosphingolipids, and amino acids were the most prevalent predictive chemical subclasses.

**Conclusions and Implications**: Significant heterogeneity of participant characteristics and methods employed among studies precluded a meta-analysis. Compounds related to lipid metabolism should be validated up to the 2nd trimester in different settings.

**Systematic review registration number:** CRD42018089985.

**Keywords**: small for gestational age, fetal growth restriction, metabolomics, prediction, gas-chromatography, mass spectrometry, vitamin D, homocysteine, lipids, fatty acids.

## STRENGHTS AND LIMITATIONS OF THIS STUDY

- To our knowledge, this is the first systematic review to assess the predictive accuracy of metabolomics for an adverse pregnancy outcome.
- Using SGA as surrogate for fetal growth restriction – just as in epidemiological investigations – improves the translational potential of metabolomics.
- Identification of techniques, types of maternal samples and chemical classes paves the way for future metabolomics investigations on fetal growth patterns.
- Available data could not support a meta-analysis; further studies should include accuracy measures of individual metabolites or chemical subclasses in predicting SGA.

**ORIGINAL PROTOCOL:** Leite DFB, Morillon A-C, Melo Júnior EF, *et al*. Metabolomics for predicting fetal growth restriction: protocol for a systematic review and meta-analysis. *BMJ Open* 2018;8:e022743. doi:10.1136/bmjopen-2018-022743.

## INTRODUCTION

Fetal growth restriction (FGR) and small for gestational age (SGA) infants are major concerns in modern obstetrics. [1–3] SGA is commonly used as a proxy for FGR, [4] despite the subtle differences between these two pathological conditions. The prevalence of both varies according to criteria applied and on the population and setting, although it reaches as much as 25% in low and middle-income countries. [5] SGA newborns may have adverse health effects, such as stillbirth, [4] perinatal asphyxia, [6] impaired neurodevelopment, [7] and increased cardiovascular risk. [8,9] To date, there are no robust prediction tools for SGA using clinical factors, [10,11] ultrasound data, [12,13] or placental biomarkers. [14]

For hypothesis generating or validation purposes, metabolomics is a novel area of biomarker, discovery, development and clinical diagnostics in translational medicine. [15,16] Metabolomics is the study of all metabolites [15,16] in a given sample, i.e. low molecular weight compounds (50-2000 Da) that are intermediates of biochemical reactions and metabolic pathways, considered to directly reflect cellular activity and phenotype. [15,16] Recent studies have evaluated the pathophysiology [17–20] of SGA with metabolomics. However, little is known about the potential of metabolomics to identify predictive compounds of SGA.

Since metabolomics can identify multiple metabolites from low volume samples, and create a model from a collection of these samples, [15] it is a promising technology for hypothesis generation in a heterogeneous condition such as SGA. The prediction of SGA in pregnancy would help refer women to specialized care facilities, improving maternal and neonatal outcomes. [21,22]

In this context, our review question was “What is the accuracy of metabolomics for predicting FGR?”. Then, the main objective of this systematic

review was to assess the accuracy of metabolomics techniques in predicting SGA. As a secondary aim, we intended to determine which metabolites are predictive of this condition.

## METHODS

The protocol for this systematic review was published previously. [23] This study follows international guidelines for transparency (PROSPERO, CRD 42018089985) and respects the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement. [24] For this systematic review, ethical approval was unnecessary.

### Literature Search Strategy

Two independent researchers (DFBL and ACM) assessed 11 electronic databases (PubMed, EMBASE, Latin American and Caribbean Health Sciences Literature (LILACS), Scientific Electronic Library Online (Scielo), Health Technology Assessment (HTA), Database of Abstracts of Reviews of Effects (DARE), Aggressive Research Intelligence Facility (ARIF), Cumulative Index of Nursing and Allied Health Literature (CINAHL), Maternity and Infant Care (MIDIRS), Scopus, and Web of Science) and grey literature. There were no limits or language constraints; the search strategy covered published documents between 1998 and 2018. Keywords 'small for gestational age', 'metabolomics', 'prediction', 'antenatal', and variations of each, were combined with Boolean operators depending on each database requirements. The full EMBASE literature search was, as follows: ('fetal growth retardation' OR 'fetal growth restriction' OR 'intrauterine growth restriction' OR 'intrauterine growth retardation' OR 'small for gestational age') AND ('metabolomic*' OR 'metabonomic*'

OR 'metabolit* 'H NMR' OR 'proton NMR' OR 'proton nuclear magnetic resonance' OR 'liquid chromatogra*' OR 'gas chromatogra*' OR 'UPLC' OR 'ultra-performance' OR 'ultra performance liquid chromatograph*') AND ('pregnan*' OR 'antenat*' OR 'ante nat*' OR 'prenat*' OR 'pre nat*') AND ('screen*' OR 'predict*' OR 'metabolic profil*'). Please check Supplementary Material 1 for more details.

**Outcomes and subgroup analysis**

The primary outcome was SGA, as a surrogate for FGR and defined as birthweight <10$^{th}$ centile, by population-based or customized charts. Secondary outcomes were birthweight ≤5th or ≤3rd centile.

The intended subgroup analysis comprised: type of metabolomics technique applied (nuclear magnetic resonance, NMR; gas or liquid chromatography coupled with mass spectrometry, GC-MS or LC-MS respectively); maternal health status before pregnancy (women with *versus* without any chronic health condition); type of SGA suspected during pregnancy (early *versus* late SGA); and type of pregnancy (singleton *versus* multiple pregnancy).

**Selection Criteria of Studies, Data Collection and Analysis**

Cohort or case-control studies were included if maternal samples were collected before the clinical diagnosis of SGA, if any metabolomics technique was applied, and if the results of SGA were presented. Articles presenting data from the same research project but analyzing distinct metabolites or showing data from different countries were included. Studies were excluded (i) according to study design; (ii) if they had not applied any metabolomics technique; (iii) if they were only experimental studies; (iv) if it was not possible to extract data on SGA; (v) or if they presented

duplicate data, in which case the most complete publication was included for final analysis.

Two researchers (DFBL and ACM) independently selected studies, extracted data and discussed discrepancies. One additional reviewer (EFMJ or RTS) helped to decide, by majority, when no consensus was reached.

Piloted standardized forms were applied for data extraction, including pregnancy characteristics and experimental details. The Human Metabolome Database (HMDB) [25] and the Kyoto Encyclopedia of Genes and Genomes [26] were used for matching chemical class and metabolic pathways of each metabolite, respectively.

**Risk of bias and Assessment of concerns regarding applicability**

Two researchers (DFBL and ACM) independently evaluated individual studies using the QUADAS-2 tool. [27] One of the third reviewers (EFMJ, or RTS) helped in decision-making when no consensus was achieved.

Each study was classified as high, low, or unclear risk of bias in four Domains (Patient Selection, Index Test, Reference Standard, and Flow and Timing), and as high, low, or unclear concerns regarding applicability in the first three Domains. We did not consider two signaling questions ("Was a case-control design avoided?", "Was there an appropriate interval between the index test and reference standard?"). The nested case-control design was an inclusion criterion and maternal samples should have been collected during pregnancy, i.e. before the SGA diagnosis. Studies were considered 'low risk', for example, (i) if pregnancy or neonatal complications were not excluded in just one group of participants or data on participant selection had been provided; (ii) if methods for sample preparation and

interpretation were standardized or metabolite threshold was defined before the experiments (for targeted analysis); (iii) if the adequacy and reasons for choosing the reference birthweight chart had been explained; or, (iv) if large for gestational age babies had been excluded from the final comparative analysis.

**Data synthesis**

A quantitative summary of data was performed when any predictive accuracy measures could be extracted. Authors were contacted to provide additional information, when necessary. However, only Delplancke et al [28] replied. The estimation of likelihood ratios and hierarchical summary receiver operator characteristic curve [29] were planned, as well as assessment of heterogeneity and publication bias. [30] However, due to lack of data, a meta-analysis could not be performed.

**Patient and Public Involvement**

There was no patient or public involvement in conducting this systematic review.

**Data Availability Statement**

All data relevant to this systematic review are included in this manuscript - in the article or uploaded as supplementary information. There are no individual patient identifiable data.

# RESULTS

## Literature search characteristics

The literature search for this systematic review was performed in February 2018, and re-run in November 2018. A total of 9,181 references were retrieved (Figure 1). After the removal of duplicate records (n=273), title and abstract screening, and analysis of the remaining 148 full-text articles, 15 articles were included. [17,18,28,31–42] See Supplementary Material 2 for excluded studies.

**Characteristics of the included studies**

The characteristics of the included studies are shown in Table 1. The prevalence of SGA ranged from 7.3% [33] to 21.5% in cohort studies. [28] There were no studies using a birthweight ≤3rd centile for a definition of SGA. The time interval between initial participant enrollment and publication varied from three [17] to 54 years, [40] although these data were unclear in 38% of the reports. [18,28,32,33,37] In nested case-control studies, participants were matched by maternal age, [17,18,38,42] ethnicity, [17,18,42] parity, [38] body mass index, [17,18,42] or infant gender. [18,38]

Participant characteristics varied between studies. Regarding gestational age at assessment, samples were collected in the 2nd trimester in one half of the studies. [17,18,33,35,37,39,42] In three reports, women were assessed at least twice. [34,38,41] In one study, maternal blood was drawn either in the 1st or 2nd trimester; [40] and in another three studies, only samples from the 3rd trimester were considered. [28,36,41] In the latter case, maternal hair was divided according to length, allowing evaluation of 2nd and 3rd trimester metabolites. [28] Studies considering the 5th centile as the cutoff, sampled women in the 1st trimester. [31,32] Twin pregnancy was a clear exclusion criterion in most studies. [17,18,31,33–35,37,40–42] Pregnancy aided by assisted reproduction [18,37] or women with pre-existing conditions [17,18,35,37,42] were also excluded, although these data were

incompletely reported. [28,32,36,38,39,41] When both nulliparous and multiparous women were enrolled, there was no data analysis according to parity. Half of the studies considered term deliveries exclusively, [18,28,36,38–41] and the remaining studies did not differentiate results according to gestational age at birth.

Regarding clinical risk factors for SGA, only one paper mentioned a previous history of SGA, but findings were not adjusted for this variable. [32] All studies, except one, [28] cited participant smoking status. The rate of smoking habit ranged from 2.4% [18] to 47.5%. [40] It is important to note that Gernand et al [40] analyzed samples from women recruited between 1959 and 1965, when smoking while pregnant was encouraged, which explains the high rate of smoking participants. The duration of smoking or any differences in birthweight (absolute measures or centiles) were not clearly stated. Although more prevalent in SGA pregnancies, results did not change with this variable control. [31,32,35,37,40] Only Gong et al [41] mentioned the suspicion of SGA in pregnancy, exhibiting decreasing abdominal circumference growth velocity between 20-36 wks. However, on final analysis, these babies were grouped with infants not suspected during pregnancy.

Table 1. Main characteristics of included studies

| Authors, year | Country, year of participants enrolment | Study design | Affected/ non-affected | Gestational age at assessment | Type of pregnancy | Parity | Birthweight curve |
|---|---|---|---|---|---|---|---|
| **Outcome: SGA <5th centile** | | | | | | | |
| Costet N et al, 2011 | France, 2002-2006 (PELAGIE Cohort) | Nested case-control | 134/ 399 | 11w | Single pregnancy | Nulliparous and parous women, unclear proportions | Customized curve |
| Ertl R et al, 2012 | United Kingdom [a] | Nested case-control | 150/ 1,000 | $11^{+0}$-$13^{+6}$w | Unclear | 55.3% nulliparous in SGA group, 48.1% nulliparous in control group | Population-based charts |
| **Outcome: SGA <10th centile** | | | | | | | |
| Grandone E et al, 2006 | Italy [a] | Cohort | 31/ 393 | 17.1 ± 1.2w[b] (mean) | Single pregnancy; no maternal pre-existing conditions | Unclear | Population-based charts |
| van Eijsden M et al, 2008 | Netherlands, 2003-2004 (ABCD Study) | Cohort | 429/ 3275 | 13.5 ± 3.3w (mean) | Term deliveries, no diabetes or hypertension | 57.6% nulliparous | Population-based charts |
| Horgan RP et al, 2011 | Australia, 2008-2011 (SCOPE Cohort) | Nested case-control | 40/ 40 | 14-16w | Single pregnancy; no other pregnancy complications | Nulliparous | Customized curve |
| Gernand AD et al, 2013 | United States, 1959-1965 (Collaborative Perinatal Project) | Nested case-control | 395/ 1751 | ≤26w | Single pregnancy; term deliveries | Parous women | Population-based charts |
| Sulek K et al, 2014 | Singapore [a] (GUSTO Study) | Nested case-control | 41/ 42 | 26-28w | Single pregnancy; term deliveries; no maternal pre-existing conditions | Nulliparous and parous women, unclear proportions | Population-based charts |
| Choi R et al, 2016 | South Korea, 2012-2013 | Cohort | 39/ 217 | 1st, 2nd or 3rd trimester | Single pregnancies | Nulliparous and parous women, unclear | Population-based charts |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | | proportions | |
| Kiely ME et al, 2016 | Ireland, 2008-2011 (SCOPE Cohort) | Cohort | 190/ 1578 | 14-16w | Single pregnancy; no maternal pre-existing conditions | Nulliparous | Customized curve |
| Ong YL et al, 2016 | Singapore [a] (GUSTO Study) | Cohort | 83/ 827 | 26-28w | Single pregnancy; no maternal chronic illness | 43.5% nulliparous | Population-based charts |
| Wang Y et al, 2016 | Taiwan, 2000-2001 (Taiwan Maternal and Infant Cohort Study) | Cohort | 35/ 188 | 3rd trimester | Unclear; term deliveries | 43% nulliparous | Population-based charts |
| Delplancke TDJ et al, 2018 | New Zealand [a] | Cohort | 20/ 73 | 34-37w | Unclear; term deliveries | Unclear | Customized curve |
| Luthra G et al, 2018 | United States, 2010-2012 (TIDES Study) | Nested case-control | 53/ 106 | 1st and 2nd trimester | Single pregnancies; term deliveries | 68% nulliparous | Customized curve |
| Gong S et al, 2018 | United Kingdom, 2008-2012 (POP study) | Nested case-control | 162/259 | 36w | Single pregnancies; term deliveries | Nulliparous | Customized curve |
| Morillon A-C et al, 2018 | 2008-2011 (SCOPE Study) | Nested case-control | 40/40 | 20w | Single pregnancies | Nulliparous | Customized curve |

[a] Unclear period of participant recruitment. [b] Mean for all study participants.

## Subgroup analysis

Due to unavailable data, the only subgroup analysis performed was related to the metabolomics approach applied (Table 2). There was no mention of adherence to metabolomics reporting data guidelines. LC-MS was the leading technique used. Three studies have investigated metabolites related to environmental exposure, from contaminated water, [31] consumer products,[36] or pesticides, [42] while others have analyzed endogenous compounds. [32–35,37–40] Only Luthra et al conducted a biomarker validation study, [38] while Gong et al [41] chose to analyze the top ten statistically different metabolites according to infant sex.

Maternal blood was the most common biological sample analyzed by LC-MS in all studies, [17,32,34–37,39–41] except for one, which used GC-MS.[39] Maternal urine was analyzed by NMR, [38] GC-MS [36] or LC-MS. [42] There was only one report using amniotic fluid [33] and two using maternal hair, [18,28] all applying GC-MS. The period of laboratory analysis was rarely specified, which made it impossible to estimate total time of sample storage.

Untargeted studies reported diverse metabolic features. Authors matched the peaks with an in-house library [18,28] or HMDB-related database. [17,42] Horgan et al [17] found 785 compounds both in maternal and newborn samples; their predictive model included 19 metabolites (only five could be putatively identified, Table 2) and used 2$^{nd}$ trimester maternal blood. Sulek et al [18] and Delplancke et al [28] prepared and analyzed samples with GC-MS using similar protocols. Sulek et al [18] identified 32 statistically different chromatographic features from which they built a predictive model using five metabolites, including two fatty acids (2-methyloctadecanoate and margarate). In contrast, Delplancke et al, [28] identified 198 metabolites, including three fatty acids (margaric, pentadecanoic, and myristic

acid) showing significantly higher levels in SGA cases, when 2nd trimester maternal hair segments were studied.

Table 2. Subgroup analysis of included studies according to which metabolomics technique was applied.

| Authors/ year | Metabolomics Technique | Maternal sample/ Storage temperature | Prediction model* | Targeted compounds | Coefficient of variation/ Limits of quantitation | Predictive compounds | Sensitivity/ Specificity | AUC |
|---|---|---|---|---|---|---|---|---|
| **Nuclear magnetic resonance** | | | | | | | | |
| Luthra G et al, 2018 | $^{1}$H-NMR 1D NOESY with pre-saturation and homonuclear 2D *J*-resolved at 300 K<br>Bruker 600 MHz Advance III HD spectrometer | Urine/ -80°C | Targeted | Tyrosine, acetate, formate, trimethylamine | NA | None | | |
| **Gas chromatography coupled to mass spectrometry** | | | | | | | | |
| Costet N et al, 2011 | GC-MS<br>Simple head space SPME-Capillary GC | Urine/ -20°C | Targeted | Trichloroacetic acid | <5%/ 0.01mg/L | None | 0.1/ 0.93 | |
| Sulek K et al, 2014 | GC-MS<br>Thermo Trace GC Ultra system coupled to ISQ mass selective detector<br>Capillary GC column: Phenomenex ZB-1701 (30 m x 250 µm id x 0.15 µm with 5 m guard column) | Hair/ -20°C | Untargeted | NA | NA | ↓ Lactate<br>↓ Levulinate<br>↑2-methyloctadecanate<br>↑Tyrosine<br>↓ Margarate | | 0.998 |
| Delplancke TDJ et al, 2018 | GC-MS:<br>Agilent 7890B gas chromatograph, capillary column ZB-1701 (30m x 250µm id x 0.15µm with 5m guard column)<br>5977 A mass spectrometer, electron impact ionisation | Hair/ -20°C | Untargeted | NA | NA | ↑ Margaric acid<br>↑ Pentadecanoic acid<br>↑ Myristic acid[c] | | 0.72<br>0.73<br>0.73 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Liquid chromatography coupled to mass spectrometry** | | | | | | | | |
| Grandone E et al, 2006 | LC-MS/MS triple quadrupole Applera API 3000, TurboIonSpray ionisation | Amniotic fluid/ -80°C | Targeted | Homocysteine | Unclear | ↑Homocystine (1,29µM; 1,05-1,52M) | | |
| Horgan RP et al, 2011 | UPLC- MS/MS Thermo Fisher LTQ Orbitrap, ESI | Plasma/ -80°C | Untargeted | NA | NA | Hexacosanedioic acid, diglyceride, lyso-phosphocholine, sphinganine 1-phosphate; sphingosine 1-phosphate[d] | | 0.90 |
| Ertl R et al, 2012 | HPLC- MS/MS Shimadzu Prominence HPLC system with a column Phenomenex Luna C8 3 x 50 mm; AbSciex API-5000 triple quadrupole, ESI | Serum/ -80°C | Targeted | $25(OH)D_2$; $25(OH)D_3$ | 6.3%[a], 6.6%[b] ($D_2$); 6.5%[a], 7.3%[b] ($D_3$)/ unclear | ↓25,OH) Vitamin D (12.16ng/mL; 8.09-20.54ng/mL) | 0.72/ 0.45 | |
| Gernand AD et al, 2013 | LC-MS/MS | Serum/ -20°C | Targeted | $25(OH)D_2$; $25(OH)D_3$ | 8.2%[a] ($D_2$) 5.9%[a] ($D_3$)/ <1ng/mL | None | 0.39/ 0.66 | |
| Choi R et al, 2016 | HPLC- MS/MS Waters HPLC system, Applied Biosystems API-4000 MS/MS mass spectrometer | Serum/ -20°C | Targeted | Methylmalonic acid; homocysteine | <10%[a]; <10%[b]/ Unclear | None; | | |
| Kiely ME et al, 2016 | UPLC- MS/MS Waters Acquity UPLS system, Waters Triple Quadrupole TQD mass spectrometer | Serum/ -80°C | Targeted | $25(OH)D_2$; $25(OH)D_3$; 3-epi-$25(OH)D_3$. | <6%[a]; <5%[b]/ 0.57ng/mL ($D_2$); 0.26ng/mL ($D_3$), 0,41ng/mL (epi-$D_3$) | None | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Ong YL et al, 2016 | LC-MS/MS Applied Biosystems ThermoHypersil BDS C8 reverse-phase column | Plasma/ Unclear | Targeted | 25(OH)$D_{2;}$ 25(OH)$D_3$ | ≤10,3%[a,b]/ <1,6ng/mL | None | 0.12/ 0.87 |
| Wang Y et al, 2016 | LC-MS Agilent HPLC system, Applied Biosystems Sciex API-4000 triple quadrupole mass spectrometer | Serum/ Unclear | Targeted | PFOA; long-chain PFCA | 0,83-7,94%[a]; 1,57-24,7%[b]/ 0,07-0,45ng/mL[e] | PFDeA (OR 3,4; 95%CI 1,07-9,19), PFUnDA (OR 1,83; 95% CI 1,01-3,32)[f] | |
| Gong S et al, 2018 | LC-MS/MS Shimadzu UK Limited UPLC system, ACE Excel 2 C18-PFP LC-column; Thermo Fisher Scientific Exactive orbitrap mass spectrometer | Serum/ Unclear | Untargeted | NA | | ↑N1,N12-diacetylspermine[f] | |
| Morillon A-C et al, 2018 | UPLC- MS/MS Waters Acquity UPLS system, Waters Synapt G2-S mass spectrometer | Urine/-80oC | Untargeted | NA | | None | |
| Others | | | | | | | |
| van Eijsden M et al, 2008 | GC-FID Solid phase extraction SPE, Capillary GC | Plasma/ -80ºC | Semi-targeted, Lipid extraction | Elaidic, linoleic, alfa-linolenic, eicosatetraenoic, EPA, DPA, DHA DGLA, AA, Adrenic, and Osbond acids | ≤2 - 22%[b]/ Unclear | ↓ Eicosatetraenoic acid (OR 1,5; 95% CI 1,07-2,11); ↓DPA (OR 1,45; 95% CI 1,06-2,) | |

[a]Intra-assay and [b]inter-assay coefficients of variation. [c]These metabolites were found in 2nd trimester hair segments. [d]And more 14 metabolites that could not be identified certain based on chromatographic peak and mass: Phenylacetylglutamine or formyl-N-acetyl-5-methroxykynurenamine; leucyl-leucyl-norleucine or sphingosine 1-phosphate; cervonyl carnitine and/or 1-alpha,25-dihydroxy-18-oxocholecalciferol; (15Z)-tetracosenoic acid or 10,13-dimethyl-11-docosyne-10,13-diol or trans-selacholeic acid; pencosenoic acid or cyclohexyl acetate or octanoic acid or methyl-heptenoic acid or 4-hydroxy-2-octenal or DL-2-aminooctanoic acid or 3-amino-octanoic acid; hydroxybutyrate or hydroxy-methylpropanoate or methyl methoxyacetate; lysophosphocoline and phosphocoline (more than 10 hits); phosphocoline (more than 20 hits); phosphocoline or ubiquinone-8; acetylleucil-leucil-norleucinal or oleoylglycerone phosphate or LPA(0:0/18:2(9Z,12Z)) or 1-16:1-lysoPE or phosphocoline(O-11:1(10E)/2:0) or (3s)-3,4-Di-N-hexanoyloxybutyl-1-phosphocoline or N-(3-hydroxy-propyl) arachidonoyl amine or N-methyl N-(2-hydroxy-ethyl) arachidonoyl amine or similar; lysophosphocholine (16:1) or cervonyl carnitine; preganediol-3-glucuronide or 3-alpha,20-alpha-dihydroxy-5-beta-pregnane-3-glucuronide; 6-hydroxyshingosine or

(4OH,8Z,t18:1) sphingosine or 15-methyl-15-prostaglandin D2 or 15-R-prostaglandin E2 methylester. [e]Values for all studied metabolites. [f]Predictive compounds only for female babies.

AUC: area under the receiver operating characteristic curve; $^{1}$H-NMR: hydrogen nuclear magnetic resonance; NOESY: nuclear Overhauser effect spectroscopy; GC-MS: gas chromatography coupled to mass spectrometry; SPME: solid phase micro extraction; LC-MS: liquid chromatography coupled to mass spectrometry; UPLC: ultra-performance liquid chromatography; ESI: Electrospray ionisation; FID: flame ionisation detection; PFOA: perfluorooctanoic acid; PFCA: perfluorocarboxylic acid; PFDeA: perfluorodecanoic acid; PFUnDA: perfluoroundecanoic acid; EPA: eicoisapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid; DGLA: dihomo-gama-linolenic acid; AA: arachidonic acid; OR: odds ratio; CI: confidence interval; NA: not applicable.

**Analysis of identified metabolites**

The identified compounds refer to eleven HMDB chemical classes. Fatty acids [18,28,39] comprised the most prevalent chemical class, followed by amino acids [18,33] and phosphosphingolipids [17] (Table 3).

A total of 5,974 women were assessed for vitamin D status. Results were presented as total vitamin D, [32,35,37,40] although vitamin $D_2$, $D_3$ or 3-epi-25(OH)$D_3$ [35] metabolites were measured. Results were stratified according to season of maternal sampling or latitude. Either <15ng/mL (<37.5nmol/L) [40] or <20ng/mL (<50nmol/L) [32,35,37] levels characterized vitamin D deficiency, but were statistically different in SGA pregnancies only in the 1st trimester. [32] Horgan et al found a metabolite that could represent a vitamin D derivative, but it was only predictive in combination with 18 other compounds; this model had an area under the curve (AUC) of 0.90 (optimal odds ratio (OR), 44; 95%CI 9-214). [17]

The second most frequent targeted metabolite was homocysteine, [33,34] although levels were only differentiated between normal and SGA pregnancies when measured in 2nd trimester amniotic fluid, with a multiple linear regression model $r^2$=0.012 and $p$=0.029. [33] Comparatively, the only common metabolite in 2nd trimester maternal hair was margarate, with conflicting results since it was found to be either increased (AUC 0.72, 95%CI 0.58-0.86) [28] or decreased. [18] The N1,N12-diacetylspermine and the perfluorocarboxylic acids were associated to female SGA babies, not males. The former presented a 5-fold decreased risk of SGA across quintiles. The perfluorodecanoic and perfluoroundecanoic acids presented OR of 3.14 (95%CI 1.07-9.19) and 1.83 (95%CI 1.01-3.32). [36] Tyrosine, an essential amino acid for infants, was part of the predictive model of maternal hair, combining 5 metabolites with an AUC of 0.998 (95%CI 0.992-1.0) [18]. However,

tyrosine did not predict SGA when urine samples were studied. [38] Methylmalonic acid, [34] acetate, formate, or trimethylamine, [38] did not differentiate SGA when compared to uncomplicated pregnancies ($p$>0.05).

Table 3. Predictive metabolites summarized according to their chemical class, subclass, and biological process

| Predictive metabolites | Chemical class | Chemical subclass | Metabolic pathway |
|---|---|---|---|
| Margarate | Fatty acyls | Fatty acids and conjugates | Lipid transport, metabolism, peroxidation |
| Pentadecanoic acid | Fatty acyls | Fatty acids and conjugates | Lipid transport, metabolism, peroxidation; fatty acid metabolism; fatty acid biosynthesis |
| Myristic acid | Fatty acyls | Fatty acids and conjugates | Lipid transport, metabolism, peroxidation; fatty acid metabolism; fatty acid biosynthesis |
| Eicosatetraenoic acid | Fatty acyls | Fatty acids and conjugates | Lipid transport, metabolism, peroxidation; lipid metabolism pathway |
| Docosapentaenoic acid | Fatty acyls | Fatty acids and conjugates | Lipid transport and metabolism, fatty acid metabolism, alpha linolenic acid and linoleic acid metabolisms |
| Tyrosine [a] | Carboxylic acids and derivatives | Amino acids, peptides, and analogues | Catecholamine biosynthesis; phenylalanine and tyrosine metabolism; thyroid hormone synthesis; transcription and translation |
| Homocysteine | Carboxylic acids and derivatives | Amino-acids, peptides, and analogues | Glycine and serine metabolism; methionine metabolism |
| Hexacosanedioic acid | Carboxylic acids and derivatives | Dicarboxylic acid and derivatives | Fatty acid biosynthesis |
| Sphinganine 1-phosphate | Sphingolipids | Phosphosphingolipids | Sphingolipid signalling pathway, nneuroactive ligand-receptor interaction |
| Sphingosine 1-phosphate | Sphingolipids | Phosphosphingolipids | Lipid metabolism pathway, sphingolipid metabolism |
| PFDeA | Alkyl halides | Alkyl fluorides | Not reported [b] |
| PFUnDA | Alkyl halides | Alkyl fluorides | Not reported [b] |
| 25,OH,Vitamin D | Steroids and steroids derivatives | Vitamin D and derivatives | Lipid metabolism pathway |
| Diglyceride | Glycerolipids | Diradylglycerols | Adipocytokine signaling pathway |
| Lactate | Hydroxy acids and derivatives | Alpha hydroxy acids and derivatives | Gluconeogenesis, glycogenosis types IB and IC, pyruvate metabolism, triosephosphate isomerase |
| N1,N12-diacetylspermine | Carboximidic acids and derivatives | Carboximidic acids | |
| Lyso-phosphocholine | Glycerophospholipids | Glycerophosphocholines | Not reported [b] |
| 2-methyloctadecanate | Saturated hydrocarbons | Alkanes | Not reported [b] |
| Levulinate | Keto acids and derivatives | Gamma-keto acids and derivatives | Not reported [b] |

[a] Essential amino acid for infants. [b] No human metabolic pathways reported at KEGG. PFDeA: perfluorodecanoic acid; PFUnDA: perfluoroundecanoic acid.

**Risk of bias and Applicability Concerns**

Figure 2 shows synthesized data for all included studies. See Supplementary Material 3 for individual QUADAS-2 data.

Regarding the risk of bias, all cohort studies conducted a consecutive participant inclusion. [28,33–37,39] Nested case-controls matched cases and controls randomly [33–35,41] or according to maternal and infant characteristics. [17,18,38,42] One study [41] failed to mention matching procedures ('Patient Selection' domain). Researchers were not blinded to SGA status when interpreting metabolomics results, [17,18,28,32,35–41] and thresholds of targeted metabolites were not pre-specified [31,33,36,38,39] ('Index Test' domain). Conversely, SGA identification was not influenced by the metabolomics test, although it was unclear when laboratory experiments were performed in some studies. [18,28,31,33,34,41] Birthweight charts were adequate, except for two studies. The first did not report which centile was chosen, [18] and the second used a centile designed for a different population [33] ('Reference Test' domain). Two studies were ranked as 'high risk' because not all participants were included in the analysis [31,37] ('Flow and Timing' domain).

The QUADAS-2 tool also highlights the importance of how the findings of the included studies are suitable to the review question. In the Patient Selection domain, it was ranked as 'high applicability concerns' when infants born between the 4$^{th}$ and the 10$^{th}$ centile, but with normal abdominal circumference growth velocity, were not included in final analysis. [41] It was 'unclear' when the gestational age of maternal assessment was not standardized, [34] or was inferred by hair segment length; [28] or when few metabolites from untargeted studies were chosen for interpretation [41]

('Index Test' domain). Finally, it was 'high' when the birthweight charts applied did not correspond to the study population [18,33] ('Reference Standard' domain).

### Meta-analysis

From the 15 included studies, only three were designed for prediction purposes [17,18,42] and provided the AUC. The remaining reports described statistical differences of metabolites between SGA pregnancies and controls. [28,31–41] Accuracy measures were extracted when available (Table 2). However, due to marked heterogeneity (Tables 1 and 2) of gestational age at sampling, type of samples used, type of birthweight chart chosen, thresholds for vitamin D deficiency, metabolomics approach, and identified compounds, a meta-analysis could not be performed.

## DISCUSSION

### Main findings

In this first systematic review of metabolomics and adverse pregnancy endpoints, we presented techniques and metabolites, which were studied for the prediction of SGA. Any effect on birthweight has important implications for perinatal research, since it is related to short and long-term outcomes, [43–46] and in different generations. [47,48] Intrauterine environment influences fetal growth through epigenetic processes: altered gene expression potentially leads to distinct phenotypes. [49] Metabolomics is the most adequate approach to study this outcome, since it is most directly related to phenotype. [50]

Interpretation of metabolomics findings in pregnancy can be challenging. Firstly, maternal metabolites concentrations are influenced by placental transfer to

and from the fetus. The 'mirror effect', seen for maternal plasma and venous cord blood metabolites at birth, [51] cannot be ruled out when only maternal specimens are studied. Secondly, maternal exposure to distinct compounds may affect metabolite levels. Statistically significant differences between SGA infants and controls may not express the totality of underlying pathological pathways and have no clinical meaning. Finally, it is unclear when the processes leading to SGA are initiated. The disruption in maternal metabolism can theoretically occur at any time. In general the lower the gestational age at which the condition is suspected, the more severe the phenotype will be at birth. [52,53] Thus, the description of clinical data in translational studies must deal with all these confounding factors.

Gestational age at sampling is probably the most important parameter for prediction purposes. With timely prediction, women could be referred to specialized care, have increased surveillance, and this in turn may lead to a reduction in perinatal mortality. There are temporal changes in the maternal metabolome during pregnancy; [28,54–57] therefore, it is reasonable to expect distinctive metabolites at different stages of pregnancy, as reported here. Unfortunately, a wide or unclear definition of gestational age of sampling [34,36,38,40] render a more precise interpretation impossible, and may limit the clinical application of these results.

In contrast, gestational age at birth and birthweight centile seem to be the hallmarks of severity and prognosis of growth restriction. [6,58] Indeed, term and preterm SGA babies show distinct clinical phenotypes, and there are concerns that some babies <10$^{th}$ centile of birthweight are constitutionally small infants. [59–61] If only term deliveries are evaluated, the most severe cases of growth restriction may be potentially missed. Moreover, when term and preterm births are analyzed together, or when lower cutoffs are not specified (e.g. ≤3$^{rd}$ or ≤5$^{th}$ centile), the lack of

predictive metabolites might mean that they are distinct conditions. Thus, we hypothesize that the predictive performance of metabolomics may be improved if data is analyzed by gestational age at delivery, and by different cutoffs of birthweight centiles.

Evidence suggests that tobacco smoke has an impact on birthweight, [62–64] although it is uncertain how and when fetal growth is impaired. It is possibly related to oxidative stress, [65] and both maternal and fetal metabolism may be disturbed at delivery. [66,67] Studies that were included did not investigate cigarette-related chemicals or quantify exposure to tobacco smoke. Therefore, no relationship between SGA and tobacco was found. Hence, we suggest that tobacco interferes with ongoing metabolic pathological processes, or its disturbance is related to additional metabolic pathways other than the one examined by the included studies.

**Subgroup and metabolite findings**

No reports have explored data on any maternal chronic condition, suspicion of SGA in pregnancy, or number of fetuses. The lack of clear statements about participant selection have hindered data interpretation and precluded these analyses.

The majority of included studies performed a targeted approach, i.e. a hypothesis-testing evaluation, [16,50] driven by epidemiological or experimental data regarding SGA newborns. None of the targeted metabolites [31–40] were in common with those found by 'hypothesis-generating' metabolic profiling [17,18,28,41,42] investigations. This reinforces the suggestion that various maternal metabolic pathways may be triggered by the SGA condition, and be detected by different biological samples. However, since blood is a very complex sample and GC-MS only

evaluates volatile molecules, [50] therefore our findings may be biased by study methodologies.

Untargeted studies, as expected, have characterized several metabolites that may be validated in future investigations. Nine lipids and fatty acid metabolites, [17,18,28,39] two amino acids, [18,33] and a steroid [17,32] have been identified as potential biomarkers of SGA.

All lipid-related metabolites identified are intermediates for energy storage and breakdown. Most metabolites were found in maternal blood [17] or hair of the SGA group. [18,28] Blood levels of saturated and monounsaturated non-esterified fatty acids apparently remain stable throughout pregnancy, while long chain polyunsaturated fatty acid (DHA and EPA, for example) measurements seem to show ethnicity-related changes. [57] Experimental data shows the importance of hypoxia and oxidative stress to placental function and ultimately, to birthweight. [68,69] Findings from included studies may represent a dysregulation of lipid pathways at the placental level, but an association with maternal background is unclear. Therefore, we hypothesize that disorders of lipid metabolism may be the 'metabolic snapshot' of defective deep placentation, [70] and might reflect maternal efforts to respond to impaired fetal growth.

Recommendations on the assessment of vitamin D and cutoffs to define vitamin D deficiency in pregnancy are controversial. [71] However, vitamin D supplementation decreases SGA risk. [72] In early pregnancy, vitamin D status has been related to SGA, [73,74] which is in accordance with this review, despite the inconsistent findings. [75] There is evidence that trophoblasts actively produce and secrete vitamin D metabolites, [76] but it is not clear how they mediate fetal growth impairment. Altered hepatic gene expression and liver function in vitamin D deficient

female rats, [77] and single nucleotide polymorphisms [78] in vitamin D receptor gene have been suggested as mechanisms to be explored by a multidimensional omics approach.

Finally, homocysteine is an intermediate metabolite of the folate cycle. It is indirectly involved with DNA methylation and is a marker of folate deficiency. [79] Maternal levels rarely reach hyperhomocysteinemia limits, [80] but folate depletion [81–83] and homocysteine itself[80] are thought to be associated with a higher SGA risk. In this review, homocysteine was only statistically different in SGA pregnancies when measured in amniotic fluid, [33] although within the normal ranges proposed for 17-21 weeks. [84] Since amniocentesis is generally performed in women at higher obstetrical risk, future studies should investigate whether homocysteine in amniotic fluid represents a confounding factor or a new biomarker. [85]

**Methodological quality**

Most studies were ranked as 'low risk' of bias or applicability to the review question. However, the lack of clear descriptions of laboratory experiments, including sample preparation and storage, and blinding of the researchers to the case/control status, are major pitfalls of the included studies.

**Strengths and limitations**

To our knowledge, this is the first systematic review of metabolomics and an adverse pregnancy outcome (SGA). We presented possible biomarkers of SGA pathophysiology, metabolites implicated in lipid transport and metabolic pathways, as well as gluconeogenesis.

However, this analysis has some limitations. First, included studies showed heterogeneity, which is fundamental in systematic reviews. Indeed, there was a wide variety of participant characteristics and methods used, and not all authors provided a detailed description of methods employed. Although the Metabolomics Standard Initiative was released in 2007, [86] there is still poor adherence to guidelines. [87,88] Clear reporting [15,87,88] and data sharing in repositories are crucial steps in identifying features of interest, specifically possible biomarkers to be validated in the clinical studies. [15] Secondly, we could not perform a meta-analysis of the extracted data, impacting the translational potential of metabolomics.

Thirdly, we considered that birthweight was a surrogate measure of intrauterine development. SGA and FGR are not interchangeable concepts. However, SGA has been used as a surrogate for FGR in many clinical studies due to difficulties in defining optimal intrauterine growth: (i) FGR diagnosis relies mostly on ultrasound measurements of fetal biometry, [3,89] which in turn is subject to systematic errors; [90] (ii) intrauterine development is adaptive, rather than uniform [91] or only genetically driven; [49] (iii) growth impairment at birth better identifies adverse neonatal outcomes than during pregnancy. [58] It is recognized that changes in obstetric care occur when growth restriction is suspected, and neonatal outcomes are improved. [21,22] Thus, an accurate prediction of SGA during pregnancy will be a turning point in modern obstetrics.

## CONCLUSIONS AND IMPLICATIONS FOR PRACTICE

Using the available clinical tools, efforts to predict SGA remain disappointing. Since SGA is a heterogeneous condition, it benefits from metabolomics. This novel area of research allows analysis of numerous types of biological fluids and detects

thousands of metabolites in complex samples. [15,16,25] However, findings of this systematic review must be interpreted with caution. The type of samples used may have influenced LC-MS (2nd trimester maternal blood) and GC-MS (2nd trimester maternal hair) findings in individual studies. Furthermore, the prediction of SGA in the context of maternal disorders, suspected FGR and twin pregnancies is an open field for future metabolomics studies, and environmental exposure investigation as well.

Surprisingly, none of the studies used ≤3rd centile of birthweight as a cutoff or analyzed preterm deliveries and hypertensive syndromes. Considering our findings and the different phenotypic manifestations of SGA, we envision a better performance when (i) cutoffs other than the 10th centile are tested; (ii) data on gestational age at sampling and at birth are standardized; and (iii) other pregnancy-related syndromes are considered, especially hypertension. Thus, future metabolomics results should advance in these critical points.

Finally, all detected biomarkers were related to lipid pathways and energy metabolism. We consider that research efforts to predict SGA should focus on compounds involved in these pathways, up to the 2nd trimester of pregnancy.

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## AUTHORS CONTRIBUTIONS

DFBL and ACM have equally contributed to this report, and both are guarantors of this review. They elaborated the protocol, searched the literature, selected studies, extracted data, assessed risk of bias, and drafted the initial manuscript. RTS and EFMJ have participated in judging inclusion of studies, interpreting data, and revising the manuscript. FM have supported data extraction and have critically examined the clinical interpretation of results. ASK has discussed the quantitative data synthesis, and supervised the report writing. PNB, LCK, and JGC have supervised and approved all steps. All authors have read and agree with this submission.

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## COMPETING INTERESTS

None to declare.

## PROVENANCE AND PEER REVIEW

Not commissioned; externally peer reviewed.

## Figure captions

Figure 1. PRISMA flowchart of study identification, screening and selection. From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097. For more information, visit www.prisma-statement.org.

Figure 2. Assessment of risk of bias (A) and applicability concerns (B) of individual studies.

## Supplementary material description

Supplementary material 1 – Detailed literature search strategy.

Supplementary material 2 - List of excluded studies and reasons.

Supplementary material 3 - Individual QUADAS-2 data for all 15 included studies.

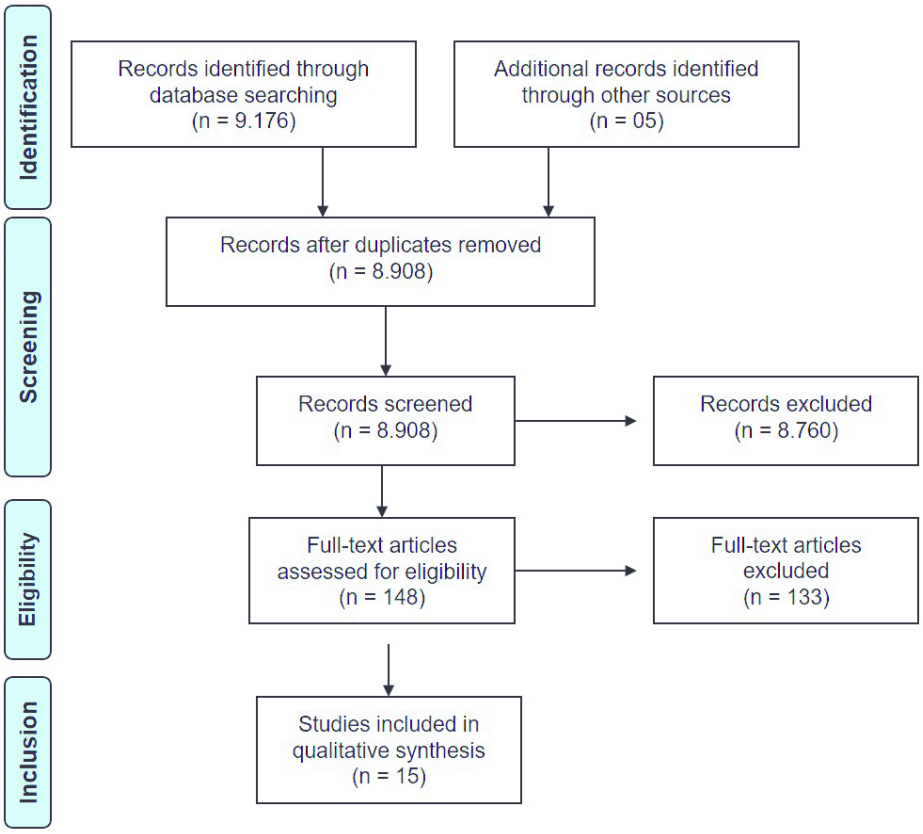


Figure 1

90x90mm (300 x 300 DPI)

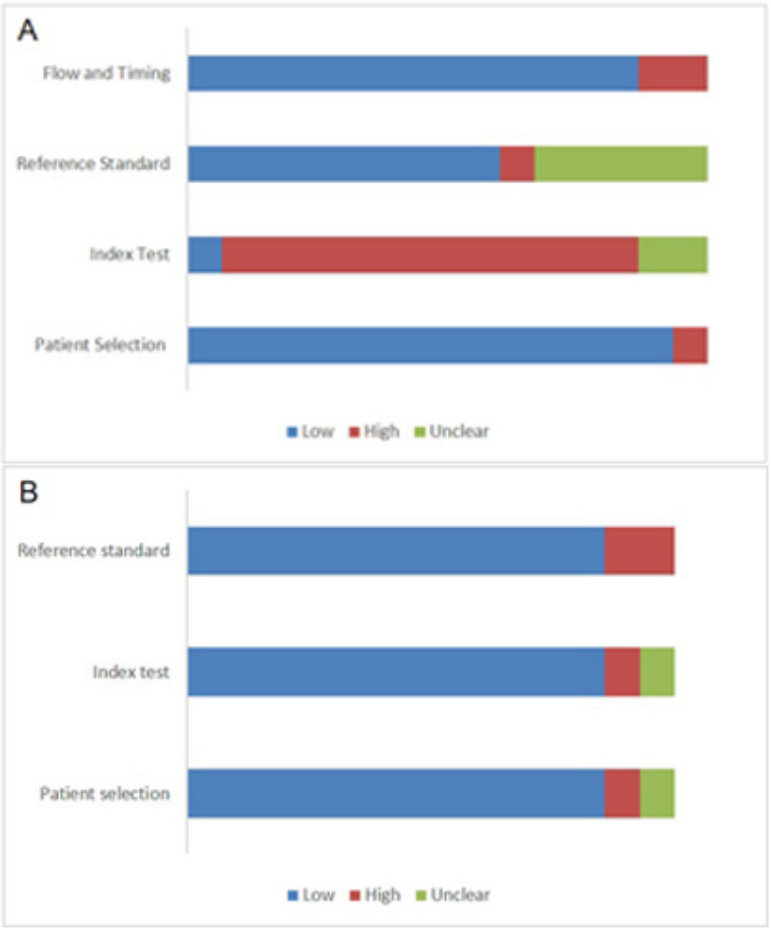


Figure 2

89x89mm (150 x 150 DPI)

**Examining the predictive accuracy of metabolomics for small for gestational age babies: a systematic review**

Debora F. B. Leite and Aude-Claire Morillon; Elias F. de Melo Junior; Renato Teixeira Souza; Fergus P McCarthy; Ali S. Khashan; Philip N. Baker; Louise C. Kenny; Jose G. Cecatti

Supplementary material 1 – Detailed literature search strategy.

| | |
|---|---|
| 1 | fetal growth retardation |
| 2 | fetal growth restriction |
| 3 | intrauterine growth restriction |
| 4 | intrauterine growth retardation |
| 5 | small for gestational age |
| 6 | #1 OR #2 OR #3 OR #4 OR #5 |
| 7 | metabolomic* |
| 8 | metabonomic* |
| 9 | metabolit* |
| 10 | H NMR |
| 11 | proton NMR |
| 12 | proton nuclear magnetic resonance |
| 13 | liquid chromatogra* |
| 14 | gas chromatogra* |
| 15 | UPLC |
| 16 | ultra-performance liquid chromatograph* |
| 17 | ultra performance liquid chromatograph* |
| 18 | #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 |
| 19 | pregnan* |
| 20 | antenat* |
| 21 | ante nat* |
| 22 | prenat* |
| 23 | pre nat* |
| 24 | #19 OR #20 OR #21 or #22 OR #23 |
| 25 | screen* |
| 26 | predict* |
| 27 | metabolic profil* |
| 28 | #25 OR #26 OR #27 |
| 29 | #6 AND #18 AND #24 AND 28 |

**Examining the predictive accuracy of metabolomics for small for gestational age babies: a systematic review**

Debora F. B. Leite & Aude-Claire Morillon, Elias F. Melo Júnior, Renato T. Souza, Fergus P. McCarthy, Ali S. Khashan, Philip N. Baker, Louise C. Kenny, José Guilherme Cecatti.

Supplementary material 2 – List of excluded studies and reasons.

| Authors/ year | Country of enrollment | Additional comments |
|---|---|---|
| *Exclusions according to study design or statistical analysis* | | |
| Barnes CM et al, 2010 | United States | Maternal samples collected at delivery. |
| Bobinski R. 2013 | Poland | Cross-sectional study. |
| Bobinski R. 2014 | Poland | Cross-sectional study. |
| Cao WC et al, 2016 | China | Cross-sectional study. The metabolomics technique was not applied. |
| Chen TT et al, 2017 | China | Cross-sectional study. |
| Cinelli et al, 2018 | Italy | |
| D'Anna R et al, 2004 | Italy | Cross-sectional study. The metabolomics technique was not applied. |
| Guo H et al, 2014 | China | Cross-sectional study. |

| | | |
|---|---|---|
| Guo J et al, 2016 | China | Cross-sectional study. |
| Maekawa R et al, 2017 | Japan | Cross-sectional study. |
| Mao D et al, 2010 | China | Cross-sectional study. |
| Miranda J et al 2018 | Spain | Cross-sectional study. |
| Powell et al, 2018 | Australia | SGA babies not suspected before birth were considered healthy infants. |
| Spanou L. et al, 2017 | Greece | Cross-sectional study. |
| Stein TP et al, 2008 | United States | Newborns with birth defects were included in the analysis. |
| Tang R et al, 2013 | China | Cross-sectional study. |
| Visentin S et al, 2017 | Italy | Maternal samples collected after clinical recognition of FGR/SGA. |
| Zhu Y et al, 2018 | China | Cross-sectional study. |
| Zota AR et al, 2009 | United States | Cross-sectional study. The metabolomics technique was not applied. |
| | | |
| *Studies that have not applied metabolomics technique* | | |
| Baker PN, 2009 | United Kingdom | |
| Berkowitz GS et al, 2004 | United States | |
| Bodnar LM et al, 2012 | United States | |

| | | |
|---|---|---|
| Braun JM et al, 2011 | United States | There is no data about FGR. |
| Cetin I et al, 2002 | Italy | |
| Chong MFF et al, 2015 | Singapore | There is no data about birth weight. |
| Colapinto CK et al, 2015 | Canada | The metabolomics technique was not applied on pregnant women's specimens. |
| Cupul-Uicab LA et al, 2013 | United States | |
| Fruscalzo A et al, 2015 | Italy | There is no data about birth weight. |
| Jusko TA et al, 2006 | United States | |
| Koepke R et al, 2004 | Mexico | |
| Lõpez-Alarcõn M et al, 2015 | Mexico | There is no data about birth weight. |
| Maruta E et al, 2017 | Japan | |
| Miranda ML et al, 2015 | United States | |
| Morley R et al, 2006 | Australia | |
| Muthayya S et al, 2006 | India | |
| Paşaoğlu H et al, 2003 | Turkey | |
| Rahman A et al, 2009 | Bangladesh | |
| Rajasingam D et al, 2009 | United Kingdom | |

| | | |
|---|---|---|
| Savitz DA et al, 2002 | United States | The metabolomics technique was not applied for pregnant women's specimens. |
| Savvidou MD et al, 2003 | United Kingdom | |
| Schneuer FJ et al, 2014 | Australia | |
| Snijder CA et al, 2013 | Netherlands | |
| Sweeney AM & Symanski E, 2007 | United States | |
| Takimoto H et al, 2007 | Japan | |
| Terrell ML et al, 2015 | United States | |
| Wei Y et al, 2017 | Bangladesh | |
| Weisskopf MG et al, 2005 | United States | |
| Whyatt RM et al, 2009 | United States | |
| Xue F et al, 2007 | United States | |
| | | |
| *Studies that have not presented specific data about FGR/SGA* | | |
| Bach CC et al, 2016 | Denmark | |
| Bachkangi P et al. | United Kingdom | |
| Bahado-Singh RO et al, 2012 | United Kingdom | |

| | |
|---|---|
| Bahado-Singh RO et al, 2015 | United Kingdom |
| Bahado-Singh RO et al, 2017 | United Kingdom |
| Bentley-Lewis R, 2015 | United States |
| Braun JM et al, 2009 | United States |
| Buckley JP et al, 2016 | United States |
| Cantonwine D et al, 2010 | Mexico |
| Cantonwine D et al, 2015 | United States |
| Casas M et al, 2016 | Spain |
| Castorina R et al, 2017 (a) | United States |
| Chou WC et al, 2014. | Taiwan |
| Cunha Figueiredo AC et al, 2017 | Brazil |
| Dalsager L et al, 2018 | Denmark |
| De Renzy-Martin KT. et al, 2014 | Poland |
| Debost-Legrand A et al, 2016 | France |
| Desert et al, 2015 | France |

| | |
|---|---|
| Diaz SO et al, 2011 | Portugal |
| Diaz SO et al, 2013 | Portugal |
| Dobierzewska A et al, 2017 | Chile |
| Dudzik D et al, 2015 | Spain. |
| Engström KS et al, 2010 | Bangladesh |
| Ettinger AS et al, 2017 | Canada |
| Feng L et al, 2016 | China |
| Ferguson KK et al, 2014 | United States |
| Ferguson KK et al, 2015 | United States |
| Ferguson KK et al, 2017 | United States |
| Finkelstein JL et al, 2015 | United States |
| Fischer ST et al, 2017 | United States |
| Gao H et al, 2017 | China |
| Gardner RM et al, 2011 | Bangladesh |
| Ghartey J et al, 2017 | United States |
| Graça G et al, 2010 | Portugal |

| | | |
|---|---|---|
| Graça G et al, 2012 | Portugal | |
| Graça G et al, 2012 (b) | Portugal | |
| Hogeveen M et al, 2010 | Netherlands | |
| Huang J et al, 2017 | China | |
| Kalhan SC et al, 2003 | United States | |
| Khalil AA et al, 2013 | United Kingdom | |
| Kuc S et al, 2014 | Netherlands | |
| Lenters V et al, 2013 | Greenland, Poland, Ukraine | |
| Lenters V et al, 2016 | Greenland, Poland, Ukraine | |
| Liu K et al, 2017 | China | |
| Lopez-Espinosa MJ et al, 2015 | Spain | |
| Marchlewicz EH et al, 2016 | United States | |
| Minatoya M et al, 2017 | Japan | |
| Minatoya M et al, 2017 (b) | Japan | |
| Minatoya M et al, 2018 | Japan | |
| Murphy MM et al, 2007 | Spain | There is no data about any pregnancy outcomes |

| | | |
|---|---|---|
| Odibo AO et al, 2011 | United States | |
| Pinney SE et al, 2017 | United States | |
| Polanska K et al, 2014 | Poland | |
| Polanska K et al, 2014 (b) | Poland | |
| Porter A et al, 2018 | United States | |
| Rejc B et al, 2016 | Slovenia | |
| Rijvers CAH et al, 2013 | Netherlands | |
| Robledo C et al, 2013 | United States | |
| Sachse D et al, 2012 | Norway | |
| Scholtens DM et al, 2016 | United Kingdom | |
| Shisler S et al, 2017 | United States | Not all analysis were performed with metabolomics approach. |
| Tamblyn JA et al, 2018 | Ireland | Duplicate data. Check Kiely ME et al, 2016. |
| Thomas MM et al, 2015 | New Zealand | |
| Van Lee L et al, 2015 | Singapore | |
| Virgiliou C et al, 2017 | Greece | |
| Walsh J et al, 2012 | Ireland | |

| | | |
|---|---|---|
| Wang PW et al, 2015 | Taiwan | |
| Watkins DJ et al, 2016 | United States | |
| Wolff MS et al, 2008 | United States | |
| Woods MM et al, 2017 | United States | |
| Yang P et al, 2018 | China | |
| | | |
| *Duplicate data* | | |
| Horgan R et al, 2009 | Australia | Check Horgan R et al, 2011. |
| Horgan R et al, 2011 | Australia | Check Horgan R et al, 2011. |
| Khashan AS et al, 2013 | Ireland | Check Kiely ME et al, 2016. |
| Sulek et al, 2014 | Singapore | Check Sulek et al, 2014. |

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Supplementary material 3 - Individual QUADAS-2 data for all 15 included studies.

| Studies | Risk of bias | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Patient selection | | Index test | | Reference standard | | Flow and timing | |
| | Was a consecutive or random sample of patients enrolled? | Did the study avoid inappropriate exclusions? | Were the index test results interpreted without knowledge of the results of the reference standard? | If a threshold was used, was it pre-specified? | Is the reference standard likely to correctly classify the target condition? | Were the reference standard results interpreted without knowledge of the results of the index test? | Did all patients receive the same reference standard? | Were all patients included in the analysis? |
| Grandone E et al, 2006 | Yes | Yes | Unclear | No | No | Unclear | Yes | Yes |
| van Eijsden M et al, 2008 | Yes | Yes | No | No | Yes | Yes | Yes | Yes |
| Horgan R et al, 2011 | Yes | Yes | No | Yes | Yes | Yes | Yes | Yes |
| Costet N et al, 2012 | Yes | Yes | Yes | No | Yes | Unclear | Yes | No |
| Ertl R et al, 2012 | Yes | Yes | No | Yes | Yes | Yes | Yes | Yes |
| Gernand AD et al, 2013 | Yes | Yes | No | Yes | Yes | Yes | Yes | Yes |
| Sulek K et al, 2014 | Yes | Yes | No | Yes | Unclear | Unclear | Yes | Yes |
| Choi R et al, 2016 | Yes | Yes | Unclear | Yes | Yes | Unclear | Yes | Yes |
| Kiely ME et al, 2016 | Yes | Yes | No | Yes | Yes | Yes | Yes | Yes |
| Ong YL et al, 2016 | Yes | Yes | No | Yes | Yes | Yes | Yes | No |
| Wang Y et al, 2016 | Yes | Yes | No | No | Yes | Yes | Yes | Yes |
| Delplancke TDJ et al, 2018 | Yes | Yes | No | Yes | Yes | Unclear | Yes | Yes |
| Luthra G et al, 2018 | Yes | Yes | No | No | Yes | Yes | Yes | Yes |
| Gong S et al, 2018 | No | Yes | No | No | Yes | Unclear | Yes | Yes |
| Morillon AC et al, 2018 | Yes | Yes | No | Yes | Yes | Yes | Yes | Yes |

| Studies | Applicability concerns | | |
|---|---|---|---|
| | Patient selection | Index test | Reference standard |
| | Are there concerns that the included patients do not match the review question? | Are there concerns that the index test, its conduct, or interpretation differ from the review question? | Are there concerns that the target condition as defined by the reference standard does not match the review question? |
| Grandone E et al, 2006 | No | No | Yes |
| van Eijsden M et al, 2008 | No | No | No |
| Horgan R et al, 2011 | No | No | No |
| Costet N et al, 2012 | No | No | No |
| Ertl R et al, 2012 | No | No | No |
| Gernand AD et al, 2013 | No | No | No |
| Sulek K et al, 2014 | No | No | Yes |
| Choi R et al, 2016 | Unclear | No | No |
| Kiely ME et al, 2016 | No | No | No |
| Ong YL et al, 2016 | No | No | No |
| Wang Y et al, 2016 | No | No | No |
| Delplancke TDJ et al, 2018 | No | Unclear | No |
| Luthra G et al, 2018 | No | No | No |
| Gong S et al, 2018 | Yes | Yes | No |
| Morillon AC et al, 2018 | No | No | No |

# PRISMA 2009 Checklist

## Examining the predictive accuracy of metabolomics for small for gestational age babies: a systematic review

Debora F. B. Leite & Aude-Claire Morillon, Elias F. Melo Júnior, Renato T. Souza, Fergus P. McCarthy, Ali S. Khashan, Philip N. Baker, Louise C. Kenny, José Guilherme Cecatti

| Section/topic | # | Checklist item | Reported on page # |
|---|---|---|---|
| **TITLE** | | | |
| Title | 1 | Identify the report as a systematic review, meta-analysis, or both. | 1 |
| **ABSTRACT** | | | |
| Structured summary | 2 | Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number. | 3-4 |
| **INTRODUCTION** | | | |
| Rationale | 3 | Describe the rationale for the review in the context of what is already known. | 5 |
| Objectives | 4 | Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS). | 6 |
| **METHODS** | | | |
| Protocol and registration | 5 | Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number. | 6 |
| Eligibility criteria | 6 | Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale. | 7-8 |
| Information sources | 7 | Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched. | 6-7/ 9 |
| Search | 8 | Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated. | 6-7 |
| Study selection | 9 | State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis). | 7-8 |
| Data collection process | 10 | Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators. | 8 |
| Data items | 11 | List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made. | 7-8 |
| Risk of bias in individual studies | 12 | Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis. | 8-9 |

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|---|---|---|---|
| Summary measures | 13 | State the principal summary measures (e.g., risk ratio, difference in means). | 9 |
| Synthesis of results | 14 | Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., $I^2$) for each meta-analysis. | 9 |



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|---|---|---|---|
| Risk of bias across studies | 15 | Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies). | 8-9 |
| Additional analyses | 16 | Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified. | 7 |
| **RESULTS** | | | |
| Study selection | 17 | Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram. | 9 |
| Study characteristics | 18 | For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations. | 9-13 |
| Risk of bias within studies | 19 | Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12). | 23-24 |
| Results of individual studies | 20 | For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot. | 20-22 |
| Synthesis of results | 21 | Present results of each meta-analysis done, including confidence intervals and measures of consistency. | 24 |
| Risk of bias across studies | 22 | Present results of any assessment of risk of bias across studies (see Item 15). | 9; 23-24 |
| Additional analysis | 23 | Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]). | NA |
| **DISCUSSION** | | | |
| Summary of evidence | 24 | Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers). | 24-28 |
| Limitations | 25 | Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias). | 28-29 |
| Conclusions | 26 | Provide a general interpretation of the results in the context of other evidence, and implications for future research. | 29-30 |
| **FUNDING** | | | |

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|---|---|---|---|
| Funding | 27 | Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review. | 38-39 |

*From:* Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

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